CIVILIZATION AND THE MICROBE

ARTHUR I. KENDALL

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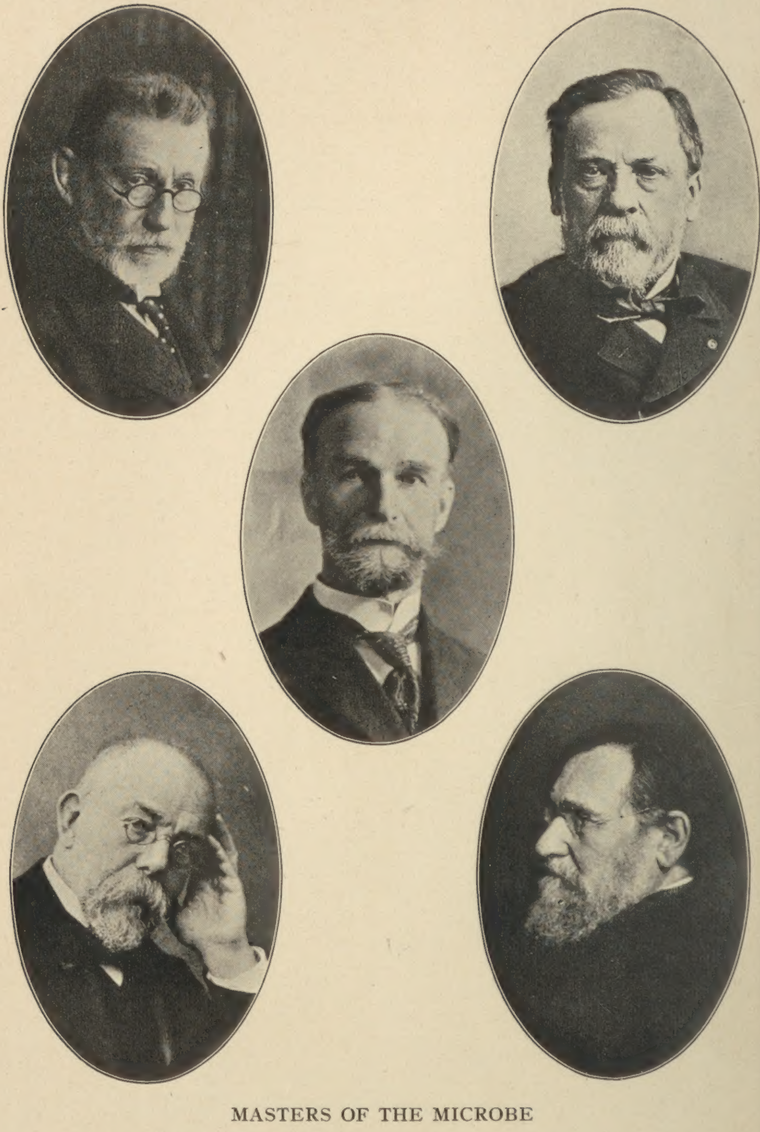

MASTERS OF THE MICROBE

EHRLICH PASTEUR

THEOBALD SMITH

KOCH METCHNIKOFF

CIVILIZATION AND THE MICROBE

BY

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WITH ILLUSTRATIONS

BOSTON AND NEW YORK
HOUGHTON MIFFLIN COMPANY
The Riverside Press Cambridge
1923

The Riverside Press
CAMBRIDGE · MASSACHUSETTS
PRINTED IN THE U.S.A.

TO

ALICE

PREFACE

My daughter Alice is a student in High School. One of the prescribed courses is General Science. The section on Bacteria left her with a vague impression of a world teeming with deadly germs awaiting an opportunity to infect mankind. It seems probable that this malignant conception of bacteria is very generally held. It is deplorable that the introduction to Science, the key to Nature's wonders, should be darkened by a vision of an unmitigated, hidden force for evil, standing squarely in opposition to man. In reality civilization owes much to the microbe.

Search for an article which should tell the story of the microbe in simple language revealed an unexpected dearth of literature upon this fascinating focal point of the sciences. To be sure, many articles and volumes have been devoted to the disease-producing bacteria, and the agricultural and industrial phases of microbic activity have received much attention, but the narrative of the bacteria with its lights and shadows does not appear to have been an attractive theme.

The manuscript which has since grown into this volume was a partly clothed skeleton of the principal features of this story, designed solely for Alice. It was planned to ask many more questions than it could possibly answer, and to stimulate her imagination, through the interrelations of the physical and biological sciences which have played and always will play such a prominent part in the field of Bacteriology.

It is with genuine pleasure I express my great indebtedness to Professor Ivan E. Wallin for the drawings of the clover plant and nodule bacteria; Professor Roy Moodie for the illustration of prehistoric bacterial infection; Professor Theodore W. Koch and Dr. Charles B. Reed for their painstaking and valuable criticisms of the manuscript; Miss M. E. Bakehouse for the drawings illustrative of the Cycle of Life, and the Sand Filter; Miss Kathryn Lanferman, my secretary, for her untiring assistance in the preparation and correction of the text; and my beloved taskmaster, Alice.

A. I. K.

CHICAGO, *August*, 1923

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INTRODUCTION

The social evolution of man from the condition of a homeless animal to an inhabitant of cities has been accomplished only at the expense of some advantages associated with nomadic life. The independent aboriginal hunter built his own lodge, made his own raiment, and fashioned his own implements. His successor of to-day has the ability to perform one small part in the complex manifestations of twentieth-century life with considerable skill. This is an inevitable result of a marked division of labor compelled by the increase in knowledge, and the inevitable complexity and volume of modern vital requirements.

The city dweller is confronted by many problems which his nomadic progenitor escaped entirely. Continuous supplies of pure food, clean milk, safe water, as well as the daily removal and disposal of the collected wastes of multitudes of peoples, create conditions which the primitive wanderer avoided very simply by moving to fresh and fruitful fields. In the solution of many of these problems, man is following in no small degree the examples worked out by Nature.

Ants and bees have a highly organized communal life. These industrious insects have evolved a very considerable division, or specialization, of individual labor in the interest of the needs of the colony. The queen perpetuates the tribe. Certain members, provided with well-developed jaws, are delegated to guard the laborers. The ants are the prototype of the primitive human husbandman; they actually enslave certain kinds of plant-lice which secrete a melliferous fluid, to serve as milch cows for the benefit of the colony. Honey bees have acquired in some wholly unknown way the secret of the storage of food unspoilable by microbic action. Honey, rich in fermentable sugar, keeps almost indefinitely. The bee gathers the honey from flowers, where the concentration of sugar with respect to the water content is relatively low; by some unrevealed process, the little toiler reverses the process, presumably by withdrawal of water, and produces thereby the highly concentrated honey. Man has as yet failed to imitate this process of dehydration without the expenditure of relatively much energy. It seems probable that the high concentration of sugar, together with the comparatively small amount of water, is the important factor in determining the stability of bee

ambrosia. A famous Frenchman, Appert, practiced this method of preservation by high sugar concentration for the first time about a century ago.

To resume the story: Man is surrounded by a microbic environment over which he has not as yet attained mastery. He is, however, slowly and laboriously acquiring practical control.

Microbic action is for the most part beneficent and essential for the maintenance of the human species, notwithstanding the very obvious opposition which a very small group of bacteria offers to the well-being of mankind. In time, the conquest of these antagonistic bacteria will be accomplished. Also, and even more important, the hidden, even unsuspected, microbic adjutants of man will be exploited to do his bidding and enrich his life. Civilization and the microbe go hand in hand, but the germ must be investigated, and the vast power locked up in the life-processes of these ever-toiling agents must be segregated and utilized to promote the prosperity and the happiness of the human race. Yes, intelligent investigation is the key to advancement. Confucius recognized this great principle of progress nearly twenty-five hundred years ago. Listen to his very words, as set forth in

that priceless Chinese classic, "The Great Learning":

The Ancients, who wished to illustrate illustrious virtue throughout the Empire, first ordered well their own States. Wishing to order well their own States, they first regulated their families. Wishing to regulate their families, they first cultivated their persons. Wishing to cultivate their persons, they first rectified their hearts. Wishing to rectify their hearts, they first sought to be sincere in their thoughts. Wishing to be sincere in their thoughts, they first extended to the utmost their knowledge. Such extension of knowledge *lay in the investigation of things.*

Things being investigated, knowledge became complete. Their knowledge being complete, their thoughts were sincere. Their thoughts being sincere, their hearts were then rectified. Their hearts being rectified, their persons were cultivated. Their persons being cultivated, their families were regulated. Their families being regulated, their States were rightly governed. Their States being rightly governed, the whole Empire was made tranquil and happy.

It cannot be, when the root is neglected, that what should spring from it will be well ordered.

CIVILIZATION AND THE MICROBE

CIVILIZATION AND THE MICROBE

CHAPTER I

What bacteria, microbes, and germs are — Their ancestry and evolution — The living earth and the function of bacteria in nature — The origin and evolution of parasitic and disease-producing bacteria — The microscope, the telescope, the theory of spontaneous generation, and the rise of the science of Bacteriology.

At the very foot of the ladder of life, simplest in structure and smallest in size of known living things, there is a group of organisms known as bacteria. Bacteria possess no green coloring matter (chlorophyll), which in the plants, through the agency of the sun's rays, permits them to build living substances from lifeless elements. Neither do bacteria possess root, stem, or leaf, nor arms, legs, head, and body. Instead of this differentiation into specialized organs for particular purposes, characteristic of higher plants and animals, all of the vital functions of bacteria, as multiplication, nutrition, and growth, are carried on in a single cell, so minute that fully fifteen millions of millions would scarcely balance an ounce weight.

The ancestral home of the bacteria seems to have been the uppermost layers of the soil, and it is here, even at the present time, that their most conspicuous and useful activities are displayed. These layers literally teem with germ life, except in the regions of perpetual snow, where, of course, conditions are unfavorable for their growth. A single particle of earth may, and frequently does, harbor hundreds of thousands of living microbes. This "living earth" is Nature's laboratory, where processes absolutely essential for the perpetuation of plant and animal life are ceaselessly carried to completion. The chemists of the living earth are bacteria. Their part in the cycle of life upon our planet is to effect a rapid decomposition of the constituents of dead animals and plants, and the products of their wastes, into simpler substances which are restored to the plant kingdom again to be rebuilt into living things. Inasmuch as some of these elements essential to life are limited in amount, this ceaseless activity of these industrious children of the living earth is essential for the very perpetuation of life upon our planet.

During the earliest days of the history of life upon this earth, before animals wandered in green meadows, bacteria were undoubtedly washed from

place to place by the torrential rains and rapidly flowing streams, but otherwise they must have remained from birth to death in very nearly the same place. With the advent of animals, however, it was inevitable that bacteria should from time to time be caught up on their bodies. Some of these bacteria apparently succeeded in adapting themselves to the new environment and permanently became parasites upon their new hosts. Considerable numbers remained indefinitely confined to the surface of the animal; others gradually became accustomed to life within the alimentary canal and other cavities where escape to the outside world and thence to other animals of the same kind was readily accomplished. There is little doubt that there were microbes among these parasites which could grow in the tissues of the animals, if the skin or other barriers were broken by wound or decreased in effectiveness by other agencies. The result must have been infection and disease, but not epidemic disease, because these parasitic forms could not of themselves *escape* from the tissues any more readily than they could *enter* the tissues, unless external factors came to their aid.

Out of the group of parasitic bacteria, however, there did develop a group of microbes, smaller in

number, but extraordinarily formidable in that their activities were definitely in opposition to those of their host. These were the progressively pathogenic bacteria. They succeeded in perfecting mechanisms for penetrating into the tissues of their victims and of escape therefrom wholly apart from any adventitious accident. The progressively pathogenic bacteria, in virtue of their tissue-penetrating powers, can and do cause progressive disease from animal to animal and from man to man. It is this group of microbes which has given to bacteria all the notoriety which they possess.

In the year 1683, about four years before the publication of Sir Isaac Newton's great work, the "Principia," a Dutch merchant, Antonj van Leeuwenhoek, who had spent his spare hours in acquiring skill in grinding spectacle lenses, placed some of these glasses in a metal tube and thus made one of the earliest, if not the earliest, compound microscope. This apparatus was very crude in comparison with the splendid instruments of to-day. It enlarged an object placed under its lenses scarcely one hundred and fifty times, or less than one tenth of the power of modern microscopes. In spite of the simplicity and imperfection of this home-made machine, however, it opened a new world to the

wondering gaze of the patient Hollander. He examined the water from pools and swamps, the tartar and scrapings from his teeth, and fecal matter from a case of dysentery. In all these he saw dimly, but definitely, extremely minute spherical, rod, and spiral-shaped "animalcules," some of which moved with considerable rapidity across his field of vision. Drawings of these minute living things, carefully preserved in the Archives of the Royal Society, reveal beyond doubt that van Leeuwenhoek had, indeed, seen bacteria, some of which are actually recognizable as true mouth microbes of peculiar and characteristic form. A new world, that of the infinitely small, had been added to the perception of man — a world relatively as insignificant in size as the stars of the heavens are stupendous. Locked up in this infinitesimal world, however, was all that Pandora's box contained, both for good and for evil. As frequently happens, not very much came of these early discoveries. The nature of the "animalcules" was wholly unknown and their significance was quite unsuspected. The imperfections of the crude microscopes available at a time when the Turks were hammering at the gates of Vienna limited observations upon microbic life to the most superficial vistas, and the lapse of a century and

a half intervened between the work of the pioneer and the exploration of the microscopic world.

This long interval of a century and a half witnessed the gradual development of that noble science of the stars, astronomy. Precisely as the discovery of the compound microscope revealed a new world of the infinitely small, so the gradual perfection of the telescope brought closer the image of objects far remote, as the stars in the sky. As each new and more powerful telescope was pointed to the starry dome in the still hour of midnight, star beyond star and world beyond world gleamed in the mighty expanse of the heavens, where hitherto dark space had resisted the eager gaze of the unarmed eye. Hundreds of thousands, and thousands of thousands, of new orbs gleamed in the firmament. How insignificant appeared man! And yet how powerful was that human brain that could bring to the aid of the eyes those instruments which would pierce the void and unfold to him the hidden splendors of those celestial giants! As the astronomers carried their observations more and more deeply into space, the course of human thought was altered profoundly by the ever-increasing grandeur and majesty of the universe. What was the origin of these remote and mighty worlds?

What all-powerful law regulated their movements, so precisely that our very conception of exact time is defined by their passage through the heavens? Are they inhabited? At this point a new epoch in the history of the human race had its beginning.

Sir Isaac Newton's formulation of the Law of Universal Gravitation, published in the "Principia," that most majestic accomplishment of the human mind, set at rest for all time speculation concerning the relations of the celestial bodies. Their movements were predictable. Their relative positions in the heavens could be stated for centuries in advance. Man had solved the fundamental problem of the movements of the universe.

Mankind then turned to speculation concerning the origin of life upon the earth. Was the spark of life brought from without, perhaps an incident in a collision with another planet? Did life originate spontaneously? Is life confined to our planet, and all the remainder of the universe gigantic but mere inorganic constituents of space? The theory of spontaneous generation alone offered opportunities for direct experimentation.

The earlier attempts to demonstrate the possibility of spontaneous generation of life were very crude, and in the light of our present knowledge

oftentimes most absurd. One noted scholar, for example, published a formula for the creation of mice. A few rags and some cheese or grain placed in a dark, quiet corner could be relied upon to produce a flourishing family of these interesting little animals where none had existed before. Some meat placed in a jar could confidently be expected to generate a swarm of maggots. Some beef tea or other food infusion would inevitably become populated with a multitude of minute animalcules, and the fœtid odor would bear witness to the success of the experiment in spontaneous generation. Many observers, however, did not believe in the doctrine of spontaneous generation.

As the years rolled by, the verbal warfare between the champions of the theory of spontaneous generation and their opponents waxed hotter and more bitter. Experiments and counter-experiments of ever-increasing refinement added greatly to the development of the science of Biology, the science of living things, without, however, furnishing a decisive answer to the problem. A time did come when even the most ardent believer in the doctrine of spontaneous generation was forced to admit that a few rags and a few crumbs of cheese were of themselves insufficient to conjure a family

of healthy mice from nothingness. The discovery was soon made that a piece of gauze tied over the mouth of a jar containing decaying meat prevented the development of maggots within. The mother blow-fly was even observed in the act of laying her eggs upon the surface of the gauze, having been attracted there by the odor of the putrefying mass inside. Maggots appeared when these eggs hatched and a new fact was in this way added to science. Thus, one by one, the grosser experiments designed to prove the doctrine of spontaneous generation failed in their original intent, although many new discoveries resulted therefrom. Mice, maggots, man, and life in general, were found to arise from preëxisting life of the same kind. The day of the microbe was close at hand.

One type of experiment in the field of spontaneous generation for many years baffled all attempts of the anti-spontaneous generationists to disprove it. It was observed that infusions prepared from meat or vegetables — clear, limpid, sweet, nutritious fluids, enclosed hermetically in flasks or bottles as soon as prepared — rapidly became turbid and foul-smelling. This took place even when they were actually sealed into glass vessels. An examination of the cloudy contents of these vessels under the

microscope revealed countless hordes of "animalcules," invisible or nearly invisible to the unarmed eye. Whence came these microscopic beings which developed in ever-increasing numbers in infusions that were so devoid of evidences of life and so pleasant when they were first prepared? Did these micro-organisms actually generate themselves, as the dogmatists claimed, or were the parent cells present, but unrecognized, in the nutrient fluids at the time they were enclosed in their glass prisons, as the biologists alleged?

Many ingenious experiments were made. Attack and counter-attack upon these experiments led to the discovery that the air harbors numerous microbes, too small to be seen by the naked eye, but made noticeable in a powerful beam of light directed through a darkened chamber. Some germs were encountered which would survive several hours' exposure to the temperature of boiling water. Others were found to be actually poisoned by the oxygen of the air, that element which is absolutely essential for the preservation of the life of most animals, plants, and for man. In the end the theory of spontaneous generation remained a discredited hypothesis. The final victory of the opponents of the doctrine that life actually may be shown to

make its appearance spontaneously from organic but lifeless substances required the perfection of the compound microscope, the rise of a new science — Bacteriology — and the labors of one of the most versatile geniuses the human race has ever known, Louis Pasteur.

Out of this conflict between empiricism and carefully executed experimentation, much good has come to mankind. There has arisen the germ theory of disease, antiseptic surgery, curative serums and antitoxins, methods for inducing resistance to microbic infection, germicides and antiseptics, all of which are of fundamental importance in communal existence. There has also arisen a gradual recognition of the extraordinarily useful and important part microbes play in the economy of nature and the conduct of everyday life. Civilization owes much to the microbe.

CHAPTER II

The forms and the appearance of bacteria — Cocci, bacilli, and spirilla — The size and weight of bacteria — How bacteria grow and multiply — Their rate of growth — Influences restraining multiplication — How bacteria move: their rate of motion — Bacterial spores and bacterial hibernation — Longevity.

BACTERIA, germs, or microbes,[1] are the smallest living things with which science has definite acquaintance. They are far too small to be seen with the naked eye. Observed under the microscope with high magnification, however, they are plainly visible. When alive, they appear as minute, colorless sacs of clear substance without any differentiation into the root, the stem, and the leaf of plants, or the arms, legs, body, and head of animals. All of the vital functions of bacteria, nutrition and growth, multiplication and function, are carried out in a single sexless cell of extremely minute proportions. For this reason bacteria are called "unicellular," or one-celled living things. Certain anilin dyes color the substance of germs deeply and make them, therefore, much more readily detectable;

[1] The exactly correct term for the micro-organisms discussed in this volume is "bacteria." *Germ* and *microbe* are used popularly and loosely to mean bacteria (in which sense they are to be interpreted in this work), or, less commonly, to mean a variety of micro-organisms not necessarily bacteria, as yeasts, moulds, and the like.

little or no more detail is brought out, however, unless very tedious methods of coloration are employed. Even then, with the exception of very delicate, threadlike processes extending from the body of the microbe, little or nothing additional is seen. These threadlike processes are called *flagella*.

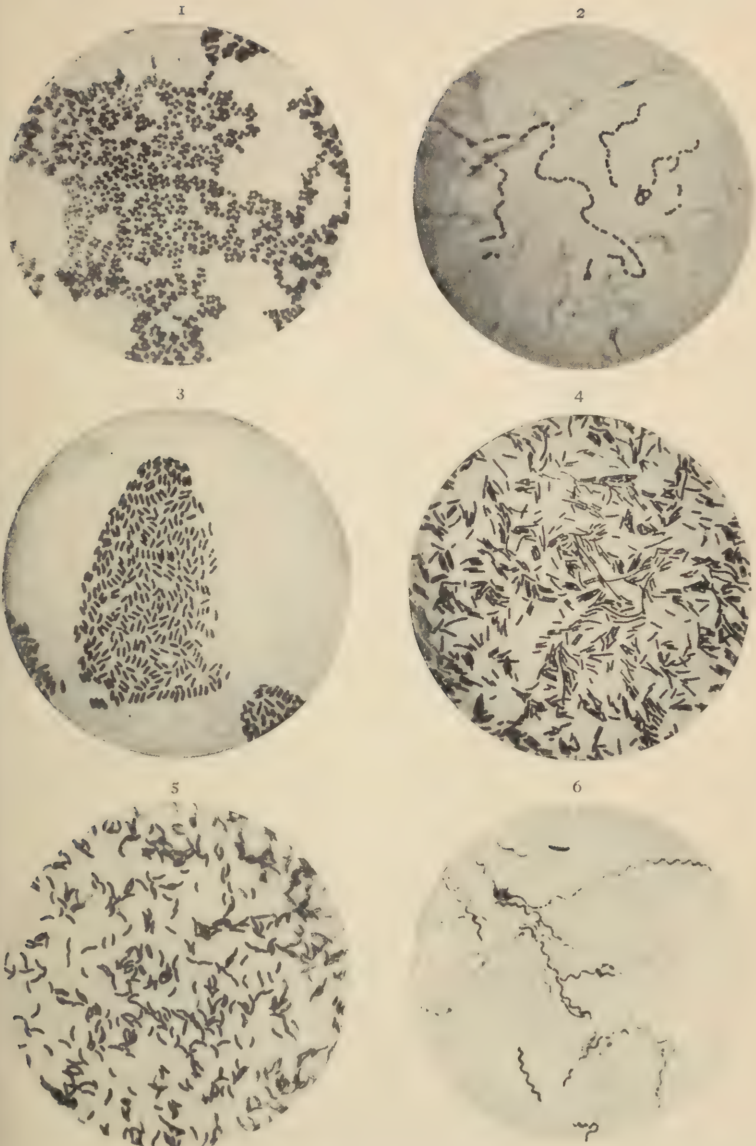
THE FORM OF BACTERIA. There are many kinds of bacteria, but all fall into one of three great groups, depending upon their form or shape. Some are spherical, like minute berries. Indeed, the name of this group is derived from a Greek word (*κόκκος*) meaning a berry. It is written *coccus*. A second group includes all bacteria which are rod-shaped. These appear under the microscope as tiny, sausage-shaped sacs, some rather long and thin, others thicker and shorter. These rod-shaped bacteria are called *bacilli*, from a Latin word (bacillum) meaning a stick or staff. A single individual of this kind is referred to as a *bacillus*. Finally, a third group is found to be elongated as the bacilli, but with this difference, that the elongated organisms are curved like a corkscrew or spring. The curvature may be very close, like a closely coiled spring, or merely a slight, sinuous twist, like half a doughnut. The curved, elongated bacteria are called *spirilla* from a

Greek word ($\sigma\pi\varepsilon\hat{\iota}\rho\alpha$) meaning a coil. A single member of the group of the spirilla is called a *spirillum*.

The number of different kinds of bacteria known to agriculture, arts and industries, medicine and hygiene, is literally in the hundreds. Since there are but three fundamental shapes or forms (cocci, bacilli, and spirilla), it follows that a mere examination of microbes under a microscope by no means renders their recognition certain. In fact, the shape of a microbe does not convey a very definite idea of its identity, excepting in very unusual instances.

The method of recognition of bacteria, therefore, is quite different from that followed in the higher plants and animals, where differences in size, shape, and arrangement of parts, members, or peculiar appendages, make classification upon appearance a comparatively simple matter. Bacteria that are identical in shape and size may be wholly unlike in their characteristics. Some may be most deadly enemies of man; others, precisely similar in appearance, may be of the utmost consequence in the economy of nature. The identification of bacteria depends not only upon their size and shape; it also requires a study of what they do in the world and a knowledge of how they do it.

Bacteriology, the name given to the study of



THE FORMS OF BACTERIA

1, 2. Two types of cocci 3, 4. Long and short bacilli

5, 6. Slightly and closely coiled spirilla

Reproduced from Fraenkel and Pfeiffer, Atlas of Bacteria

bacteria, therefore, requires the aid of chemistry and other natural sciences to help solve its problems. Inasmuch as bacteria play an important part *pro* and *con* in pure food, safe water, and other vital necessities, it is not wholly disadvantageous to require this knowledge of what bacteria do and how they do it as a part of the system of their classification.

THE SIZE OF BACTERIA. Bacteria not only require a microscope to make them individually visible; they also need a very special yardstick for their measurement. The standard of measurement for bacteria is not large; it is about one twenty-five-thousandth of an inch, or one one-thousandth of a millimeter in length. The name of this microscopic unit is the *micron*. It is designated by the Greek letter "m" (μ). The use of this bacterial gauge is of necessity conducted under the higher powers of the microscope.

Measured under the microscope with such a micrometric scale, an average-sized microbe, as the bacillus that causes typhoid fever when it grows in the tissues of man, *Bacillus typhosus*, for example measures about two microns (one twelve-thousandth of an inch) in length, and one micron

(one twenty-five thousandth of an inch) in diameter. Among the smallest of known microbes is that one called *Bacillus influenzæ*, which is so frequently found in the lungs and sputum of patients suffering from influenza: hence, the name. This microbe is scarcely half as long and rather less than half the diameter of the typhoid bacillus. Its average dimensions are 0.8 micron in length and 0.3 micron in width. At the other end of the scale is the relatively huge organism sometimes found in the alimentary canal of the cockroach, called *Bacillus bütschlii* in honor of an eminent scientist. This microbe may measure as many as fifty microns in length and five microns in diameter. Bacteria, as a rule, average about a micron in diameter.

Some approximate idea of the size of a typhoid bacillus may be gained by estimating the number which would be required to make a mass the size of a common kitchen match, which is about two and a half inches long and one eighth of an inch in diameter. Thirty-seven thousand typhoid bacilli placed end to end would just about span a distance of two and a half inches. Three thousand laid side by side with their longer dimensions parallel would just about cover a space one eighth of an inch in width. It would be a very simple calculation to

estimate the number of such bacteria required to fill a hole the size of a match.

THE WEIGHT OF BACTERIA. Knowing the measurements of bacteria, it follows that the volume may be computed; knowing the volume and the weight of the substance of the microbe as compared with an equal volume of water, the weight of the organism can be arrived at. A cylinder the size of the typhoid bacillus and 1.2 times as heavy as water would weigh almost exactly 0.000,000,002 milligrams. Five hundred millions of them, in other words, would weigh a milligram. A milligram is one thousandth of a gram, and a gram in turn is approximately one thirtieth of an ordinary ounce. Fifteen millions of millions of typhoid bacilli, therefore, would be required to balance an ounce weight.

Viewed from the standpoint of these measurements, it is not difficult to believe that bacteria are in reality about the smallest of known living things. It should be stated in this connection that a group of viruses of unknown, or nearly unknown, shape exists, which are either so small, or, less likely, so flexible, they will pass through the pores of certain kinds of stone filters. These organisms are called

"ultramicroscopic." Some of them, however — as the virus of that dread disease known as anterior poliomyelitis, or infantile paralysis — are large enough to be seen as extraordinarily minute ovoid bodies, considerably smaller than the influenza bacillus. These excepted, bacteria are very minute, the smallest of known living things.

THE RATE OF GROWTH OF BACTERIA. The nature and extent of the changes bacteria induce in the soil, in the industries, in the purification of water and sewage, and in the production of disease stand in sharpest contrast to their size. At first sight, the magnitude of their operations would seem to be wholly beyond the possibilities locked up in such minute organisms. A consideration of the phenomenal rate of growth of bacteria under favorable conditions, however, will furnish a partial explanation of their amazing capacity for work in the aggregate.

Bacteria increase in numbers by a very simple process, standing in contrast in this regard with higher plants and animals. A bacteriological individual, or cell, placed in a favorable environment of temperature, moisture, and food, elongates somewhat beyond the normal size. Then a slight

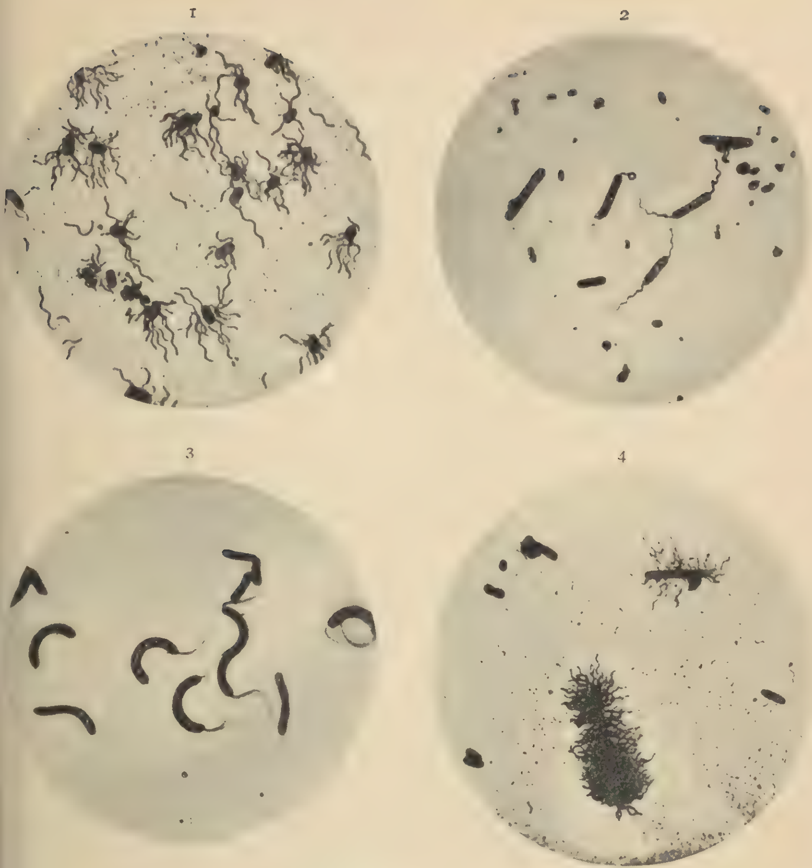
constriction appears midway between the ends of the elongated microbe, and this becomes deeper and deeper until the original, or parent cell, becomes divided into two exactly similar cells, each of the same size. Parent cell and daughter cell proceed in the same manner, each dividing again and again. Successive, fully mature generations may succeed one another at intervals as frequent as every fifteen minutes under favorable conditions in the more rapidly growing kinds. For example, the germ that causes Asiatic cholera, and many others, actually may be seen to multiply thus under the microscope. Such a rate of growth yields a stupendous progeny. Theoretically, the numbers of microbes increase thus:

At the start, one microbe; at the end of fifteen minutes, two microbes; in thirty minutes, four; in an hour, eight microbes in place of the single one at the beginning of the experiment. At the end of the second hour, the original microbe would be surrounded by a theoretical group of descendants numbering two hundred fifty-six. The third hour would yield over four thousand, and the fourth hour would in turn reveal a theoretical progeny exceeding sixty-four thousand. There are ninety-six generations, each of fifteen minutes' duration in

twenty-four hours. Mathematical geniuses will find that 2^{96}, the mathematical expression for the theoretical number of bacteria developing from a single bacterial cell, dividing each fifteen minutes, together with its descendants, will number about 8×10^{28} (78,700,000,000,000,000,000,000,000,000) in twenty-four hours. For comparison, it may be stated that there are approximately thirty millions of millions of seconds in one million years (31,536,000,000,000).

It is needless to point out that this wholly incomprehensible number of microbes never is realized, even though the process of division actually may be seen to take place under a microscope during the first hour or two.[1] Nature fortunately interposes insurmountable barriers to the realization of such an astounding theoretical rate of generation. The exhaustion of available food, the rapid accumulation of products incidental to microbic growth but inimical to the continuance of multiplication, the struggle for existence in competition with other microbes, and many other important factors all restrain bacterial growth and keep it within endurable limits.

[1] It is possible to follow the numerical development of bacteria by a method known as "plating." This is described in Chapter VII.



BACTERIA SHOWING FLAGELLA

1. Typhoid bacillus
2. Bacillus from hay infusion
3. Spirillum from water
4. Proteus bacillus

In spite of many restraints, microbic development may lead to most unexpected results.

A man may be infected with the germs of Asiatic cholera and die from the effects of their growth in his alimentary canal in less than twelve hours. Hundreds or thousands of gallons of syrup, ready for final boiling down to crystallization of sugar, may spoil in less than two days. Many hundreds of pounds of starch under proper conditions may be transformed into butyl alcohol and other substances by microbic action within a very few days. All of these instances are inseparably associated with the rapid multiplication of germs carried out where the surrounding conditions are fitted for their growth. Each microbe contributes but little; the combined activities of a multitude of feeble, separate efforts may be gigantic in the aggregate. Microbic activity properly controlled in the interest of man is an economical, ceaseless source of power.

THE RATE OF MOTION OF BACTERIA. Many bacteria possess the power of moving with considerable speed through fluid mediums in which they are suspended. The organs of locomotion are delicate, threadlike outgrowths from the body of the microbe. They are extremely thin, although they may

be two or three times the length of the organism. Careful measurements indicate they are about one tenth of a micron (one two hundred and fifty thousandth of an inch) in diameter. The rhythmic contractions of these threads, known as *flagella*, act somewhat as oars would act, and propel the microbe through the fluid with relatively considerable velocity. Painstaking studies have shown that the most active bacteria move about 0.0012 of an inch in a second. If a man should move as rapidly in proportion to his size, he could travel somewhat more than a mile in a minute. In measuring the rate of motion of bacteria, it must be remembered, of course, that the apparent speed is magnified in exactly the same ratio as the size of the organism. That is to say, if the microscope makes the image of the bacterium one thousand times as great as the actual cell itself, the apparent rate of motion with which the microbe travels is also magnified one thousand times.

BACTERIAL SPORES. Microbic life in the soil is exposed at irregular intervals to periods of drought, heat, cold, lack of suitable food, or other conditions unfavorable for continued development. Bacteria which are parasitic upon man or animals are not

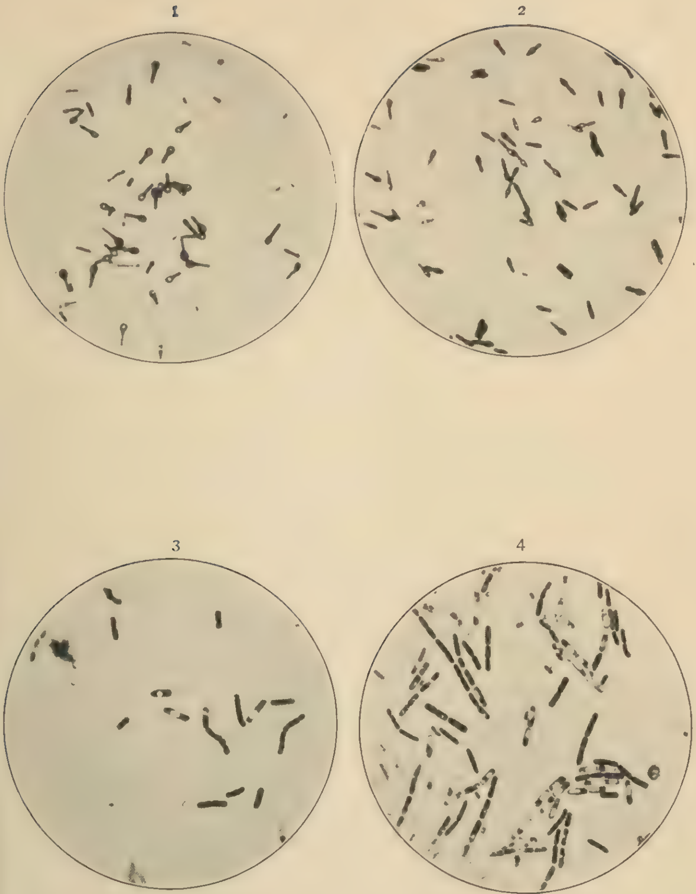
confronted, as a rule, with similar difficulties because the conditions that prevail on or in animal hosts are quite uniform.

The ordinary microbe is not endowed by nature with the ability to survive adverse conditions very long, and it is not surprising to find that those so exposed habitually have been provided with, or have acquired, a means of protection against adversity which is very effective. It is also simple. Simplicity is the hall-mark of nature.

A microbe habituated to unfavorable environmental conditions undergoes a clearly discernible change under the microscope during such periods. The cell substance becomes cloudy, and soon a large, shining mass with a dense covering or membrane appears. This is called a *spore*. One spore only forms in a single organism. It may be centrally placed, at one end, or between the center and the end. Also, its diameter may be greater or less than that of the parent cell. There is some regularity in size, shape, and position of the spore, which aids in the identification of the microbe. For example, *Bacillus tetani*, the germ of "lockjaw," habitually produces a spore at one end of the parent rod considerably larger in diameter than the original cell. The tetanus spore with parent cell attached

resembles a drumstick. Not many bacteria present this appearance at sporulation. On the other hand, the common soil germ known as the "hay bacillus," so called because it was found in cultures made from hay infusion many years ago, has a centrally placed spore, less in diameter than the parent cell.

Enough of the vital substance of the microbe enters into the spore to maintain life at a very low ebb; a sort of hibernation, as it were. The dense spore membrane and the partial removal of moisture from the spore content, two important characteristics of mature spores, increase the resistance of the residual germ substance contained within the spore walls thus fortified to heat, drying, chemical poisons, and other unfavorable conditions which would otherwise prove fatal. Thus, some spores may be kept at the temperature of boiling water for several hours without killing them. About thirty-five years ago a great bacteriologist dried some spores derived from a culture of anthrax bacilli — a microbe that causes a very fatal disease in cattle and sheep if it gains entrance to the body through wounds or cuts — upon threads, and placed these threads in hermetically sealed glass tubes. At the beginning of the experiment, enough anthrax spores were present upon an eighth of an



BACTERIAL SPORES

1. Spore at end of parent cell. *Bacillus tetani* (lockjaw bacillus)
2. Spore near end of parent cell. *Bacillus sporogenes*
3. Spore in center of parent cell. *Bacillus subtilis* (hay bacillus)
4. Spore near center of parent cell. Anthrax bacillus

inch of this infected material to kill a mouse after the organisms were grown overnight in bouillon. A piece of string, one eighth of an inch long, was carefully placed in a small amount of bouillon; this was placed in a warm, dark place for eighteen hours, and the resulting active culture of microbes contained millions and millions of virile germs. A drop of this growth placed under the skin of a mouse at the base of the tail would cause a rapidly fatal infection that caused the death of the mouse within twenty-four hours. At five-year intervals this experiment was repeated in detail. After thirty-five years it was found that an eighth of an inch of the infected thread still contained sufficient virile organisms to cause death in mice precisely as the original test caused the death of mice. Apparently drying for thirty-five years had neither killed the spores of the anthrax bacilli, nor prevented their development into active organisms, nor diminished the virulence of the descendants of these organisms to a point where mice could resist infection with one drop of the bouillon culture.

It is fortunate, indeed, that those bacteria which cause epidemic disease from man to man, as typhoid, tuberculosis, pneumonia, or from animal to animal, as glanders, hog cholera, or bovine tuber-

culosis, do not form these highly resistant bodies — spores — as a part of their life-history. All the progressively pathogenic bacteria, so far as known, are unprovided with this means of prolonging the life of the microbe away from the body of its particular host. The control of epidemic disease is obviously simplified by the absence of unusual resistance of the microbes of these diseases to drying, heat, or chemical poisons. The absence of spores in man's contagious microscopic enemies is one of the natural safeguards of civilization.

CHAPTER III

The temperature range of microbic life — Arctic snow and thermal springs — Pasteurization and the preservation of milk — Freezing and thawing and the effect of liquid air upon bacteria — Purification of water by freezing — Cold storage — Desiccation and food preservation — Effect of strong salt solutions upon bacteria — Effect of strong sugar solutions upon bacteria — Preservation of food by corning — Light without heat.

Man and those animals called "warm-blooded" differ from all other animals and plants and bacteria in one important particular. They maintain a constant, or very nearly constant, body temperature quite irrespective of the degree of heat or cold of their surroundings, provided, of course, these external conditions are not too extreme. Thus, the region of perpetual snow and ice, and the lands of tropic heat and moisture, alike are inhabited by man and animals. The human species can pass from an Arctic temperature 60° or 70° below zero Fahrenheit to a veritable oven, such as Death Valley, where the thermometer registers 120° F. in the shade, without undue interference with its ability to survive and function quite normally.

The Developmental Temperatures of Bacteria. Bacteria, on the other hand, react some-

what differently to heat and to cold. Their temperature is that of their surroundings. Among them are varieties that grow, slowly, to be sure, in the Arctic snows. Thus, in the high Alps and in the Arctics, the snow sometimes becomes quite blue or vivid red. Careful examination of these colored areas has revealed the presence of microbes that produce a blue or a red pigment incidental to their growth. Formerly, red snow was regarded with superstitious awe. Now it is heralded as one of the innumerable instances of Nature's wonderful versatility.

The bacteria of the soil for the most part grow most luxuriantly at summer heat: 60° to 70°. Those microbes which are parasitic upon, or pathogenic (disease-producing) for, man develop best at the body heat, 98.5°. The temperature of most birds is somewhat higher than that of the human body. It is not surprising, therefore, to find that the avian tubercle bacillus grows at its maximum intensity about 102°. One of the strange adaptations of bacteria to environmental conditions is found in that group known as the heat-loving (thermophilic) microbes, found in hot springs and occasionally in the alimentary canal of man and animals. Flourishing multitudes of these germs are encountered

in natural waters whose temperature ranges from 140° to 170° on the Fahrenheit scale. This temperature is quite beyond the range of human, animal, or plant endurance. The substance of the muscles, the white of egg, and other similar protein constituents of most living things undergo a change known as "heat coagulation" far below this point. Thus, egg albumen (egg white) solidifies or coagulates, if it is exposed for a short time to 140° F. All life, except bacterial spores and thermophilic bacteria, dies in an atmosphere whose temperature is materially less than 140° F. The function of heat-loving microbes is as yet an unsolved puzzle; their existence, however, and their tolerance to the heat developed in the thermal springs, are unquestionably proven.

Between the wide limits of the temperature of freezing water and that of thermal springs, then, bacteria of one kind or another live, and move, and have their being. Each and all have their respective favorable temperatures at which growth is most vigorous and most characteristic. Usually their death-point is but a few degrees in excess of their most favorable thermal developmental point.

PASTEURIZATION. Many years ago, Louis Pas-

teur, one of the greatest biologists of all time, found that perishable liquids could be protected from decomposition for considerable periods by the simple expedient of heating them for twenty or thirty minutes to a temperature of about 140° F. This degree of heat does not alter profoundly the components of the liquids, but it kills most microbes which do not form spores. A very practical application of this principle is exemplified in a useful method of restricting and partly destroying microbic contamination of milk. It is called Pasteurization in honor of Pasteur. It is carried out on a large scale in series of tanks ingeniously arranged to fill, heat, empty, and cool large amounts of milk in a very uniform manner. The process is automatic. The milk is heated to 140° F., retained at this temperature for twenty minutes, and then cooled rapidly to 40°. It keeps for three or four days without much evidence of bacterial decomposition, if it is carefully maintained at a temperature below 45° F. after the process has been completed. Spore-forming microbes are not necessarily affected by the relatively low Pasteurizing temperature. All bacteria pathogenic for man are killed, or so weakened by this process, that Pasteurized milk is quite safe for human consumption, in so far as danger from microbic infection is

concerned. It should be remarked, in passing, that human rather than bovine microbes make milk formidable as a transmitter of infection. Indeed, nearly all the pathogenic bacteria that are transmitted to man through milk are implanted in the milk by men harboring the microbes themselves. Comparatively few diseases of bovine origin are transmitted to man through milk. Careless or ignorant handling by human hands is the most prolific source of danger in the milk industry, because milk contains all the elements necessary for the nutrition of bacteria as well as man.

An illuminating experiment is frequently performed before a group of students interested in public health problems. The demonstrator provides a gallon of milk, exhibited in a glass aquarium jar, and a pint of thin mud in a suitable glass flask. A piece of white paper is placed behind the milk to furnish a good background. When all is ready, the mud is slowly poured into the milk, with continual stirring, until it is all added. At no time during the experiment can the audience see the slightest change in the color or general appearance of the milk. The secret of the success of the experiment lies in the fact that milk is entirely opaque in moderately thick layers. The lesson to be learned is that much filth may be

present in milk without any changes visible to the senses. Hundreds of millions of bacteria in each thimbleful of it may exist without any evidence of their presence. The reduction in sickness, particularly among young children, following the education of the milk producers, and the enforcement of laws designed to protect the supplies of this important food from careless or criminal neglect in handling it, is ample testimony of the dangers surrounding unsanitary milk supplies

Effect of Cold upon Bacteria. The effects of cold upon microbic activity are quite unlike the effects of heat. Even moderate degrees of heat are fatal to bacterial life, whereas the greatest cold obtainable by human and chemical agencies is surprisingly ineffective.

Physicists speak of the "absolute zero," a point at which there is absolutely no heat, where all activity ceases and where even molecular movement is stilled. This is 273° below the freezing point of water on the Centigrade scale,[1] or almost 460° below

[1] So called because the range of temperature recorded by a mercury thermometer between the point of freezing water (32° on the Fahrenheit scale), and the boiling point of water (212° on the Fahrenheit scale), is divided into one hundred equal intervals or degrees.

the zero point of the Fahrenheit scale. It has apparently never been reached upon the earth. Liquid air, however, which boils at about 300° below zero on the Fahrenheit scale, is obtainable in quantities sufficient for purposes of experimentation. The temperature at which air is a liquid is so low that mercury — that beautiful fluid metal used in thermometers — when exposed to it becomes as hard as iron. Steel becomes very brittle after a few moments' immersion.

Bacteria have been exposed in liquid air for several hours. Of course they are frozen very solidly. If they are thawed out carefully, even after six or eight hours' intensive freezing, it is found they grow with scarcely any reduction in vigor. Even microbes as formidable to man as the typhoid bacillus survive this freezing ordeal without much difficulty. Obviously bacteria are very much more resistant to thorough freezing than most other living things.

Effects of Ice Formation upon Bacteria. Experiments upon the effects of freezing bacteria have revealed a strange relationship between the nature of the substance the microbes are suspended in prior to the cooling process, and the effects of the

cooling upon their survival. For example, bacteria frozen in water, even just below the point at which ice forms, suffer a heavy mortality. If, on the contrary, they are suspended in white of egg and then frozen, the great majority survive. Alternate freezing and thawing of bacteria suspended in water will soon kill all the microbes, whereas many repetitions of freezing and warming will be without noteworthy effect upon the survival of bacteria immersed in egg white.

After much careful study it has been found that water forms crystals during the congealing process in a quiet place. Egg white forms no crystals when it freezes. The large death-rate of bacteria frozen in water — which may reach as high as eighty per cent — is due apparently to their actual crushing by the ice crystals which form and fill the container they are in. This explains the effective sterilization of water containing bacteria by the process of alternate freezing and thawing. Those microbes which escape crushing by the first formation of ice crystals succumb to successive exposures. On the other hand, bacteria enclosed in egg white, or egg albumen as it is more properly designated, congeal comfortably during the process of refrigeration and await the next warm period to resume growth.

BACTERIAL PURIFICATION OF WATER BY FREEZING. This partial purification of water by freezing has an important relation to the formation of ice on impure but quiescent river and lake water. Much of the dissolved impurity in the water, and, more important, many of the bacteria, are excluded or exterminated during the freezing process. This is one of Nature's important methods of purification, but it is not necessarily complete. It would be unwise to rely upon simple freezing as a guarantee that ice derived from contaminated waters would be safe for human use.

EFFECT OF REFRIGERATION UPON BACTERIA. It has been shown that even extreme cold, uncomplicated by other factors, is not necessarily fatal to bacterial existence. It must be realized, however, that cooling even a few degrees below the point of most luxuriant growth affects their activities quite definitely. It is well known that microbic activity in the soil ceases with the advent of cool weather. Meats are quickly decomposed in the tropics; in the polar regions they remain apparently unchanged for weeks or months at a time. From what has been said, it is quite clear that the microbic basis for rapid decay is present in these food sub-

stances. Cold does not kill bacteria. It must, however, have some influence upon their rate of growth and vital activities. The family refrigerator and cold storage are accepted, although perhaps not recognized, as practical adaptations of the effect of cold upon bacterial growth.

The very rapid growth of the microbe of Asiatic cholera and other germs, commented on in the preceding chapter, takes place only when they are kept at a temperature most favorable for their luxuriant growth and multiplication. This is the temperature of the human body for the cholera microbe — 98.5° F. A lowering of the temperature of the surroundings of the cholera germ of only a very few degrees slows up its rate of development exceedingly. At the temperature of the ice-box, about 45° F., the microbes may actually grow less in a month than they would overnight at body heat. This general relationship between temperatures below the most favorable one and the rate of growth is general among microbes.

The practical bearing of this relation between temperatures below those of maximum luxuriance of growth of bacteria and a very decided slowing of their rate of growth and general activity is found not only in the household refrigerator and cold-

storage plant, but also in the transportation of perishable foods by land and sea. Modern methods of life would be seriously interfered with were refrigeration eliminated. Much of the human food problem depends upon its successful application to bacteriological restraint.

EFFECT OF MOISTURE AND DESICCATION UPON BACTERIA. Another fact of importance in determining bacterial growth, besides a proper temperature, is that of moisture. Man and animals and bacteria all have about the same percentage of water in their substance — approximately eighty-five per cent.

It is necessary to refer at this point to the fact that small amounts of certain substances added to water will cause the mixture to become jellylike, even though a very large percentage of the mass is in reality water and may be recovered as such. Very small quantities of some of these gel-producing materials may cause the fluid to which they are added to solidify as a gel. Thus, less than half a per cent of a solution known chemically as calcium acetate (formed by the action of the acid of vinegar upon lime) will make a seventy per cent solution of grain alcohol become a semi-transparent, jellylike mass that may be cut into cubes of convenient

size. The alcohol is still alcohol, and it will burn quietly when ignited, if a lighted match is touched to one corner of this "solid alcohol." It is needless to remind the good housewife that a little gelatin added to some water will make a thick solution that will solidify to a jellylike mass when it is cool. This is a true gel. Many fruits — currants, grapes, apples, and others — contain small amounts of substances known as pectins which cause the pulp of the fruit and added water to gel.

The substance of animal, plant, and bacteria contains likewise relatively small amounts of highly complex organic compounds that hold the water content — about eighty-five per cent — in a jellylike mass. The water of the tissues is not limpid and it does not flow readily, but it is present as water and in relatively large amounts, nevertheless. Without water, all vital processes cease. It is not surprising to find, therefore, that the withdrawal of water, even in part, from the substance of bacteria, causes a cessation of their activity. The withdrawal of water may be brought about by simple drying; it can also be accomplished by suspending the microbes in strong solutions of salt or sugar. The removal of water from the substance of the microbic cell by concentrated salt or sugar solutions is not

quite so readily understood, but it is not difficult to explain, after all.

EFFECT OF STRONG SALT SOLUTIONS UPON BACTERIA. Bacterial cells, like animal or plant cells, are surrounded by a membrane or skin, which biologists call "ectoplasm." This membrane keeps the contents within from flowing away, and also confers upon the cell its shape. It possesses peculiar properties, not the least important of which is its ability to allow the free passage of water without a corresponding freedom of passage of the cell substance. Such a membrane is appropriately called "semi-permeable," because of the facts just cited. The passage of water through the semi-permeable membrane between the inside and the outside depends upon the relative attraction of the substances within and without for its direction and amount.

Salt and sugar possess a very considerable attraction for water. The housewife knows that a lump of pure salt (not salt mixed with a considerable amount of starch to make it pour readily) exposed to the air on a moist day in summer will gradually become more and more moist. Eventually it collects to itself sufficient moisture actually to dis-

solve it. A strong salt solution similarly attracts water to itself.

If bacteria, whose substance contains comparatively little salt and eighty-five per cent of water, are suddenly suspended in a strong salt solution containing comparatively little water, water will pass from within the bacterial cell through its semi-permeable membrane to the outside in response to the attraction of the salt for water without. At the same time a counter-attraction of salt from without inward is set up to equalize the pressure of the solution. The semi-permeable membrane, however, permits of only a very slow passage of salt to the cell substance, although offering little or no opposition to the passage of the water in the reverse direction. The net result is readily predicted. The cell substance becomes depleted of water; it actually dries out to a point where the life of the microbe is either reduced to a point incompatible with continued growth, or is actually extinguished.

The process known as "pickling," or "corning," better known to our ancestors than to the present generation, is an admirable example of the household application of the restraining effects of strong salt solutions upon microbic growth in the preservation of meats from decay. Strong solutions of salt-

peter (potassium nitrate) are prepared and lumps of meat destined for corning are immersed within, care being taken that the entire mass is completely submerged. The withdrawal of water from the microbes in and on the meat proceeds far enough to prevent their development. The meat, therefore, fails to decompose. The preservation of the bodies of the ancient Egyptians, Babylonians, and Peruvians by their immersion in strong solutions of nitre or other salts is another instance of desiccation and mummification.

Effect of Strong Sugar Solutions upon Bacteria. Strong sugar solutions are even more effective agents for withdrawing water than strong salt solutions. Honey, a very concentrated sugar solution prepared from the nectar of flowers by the honey bee, resists microbic decomposition for almost indefinite periods. To honey bees belongs the credit for the first demonstration of food preservation. This is ordinarily overlooked, even as most of Nature's beneficent processes are overlooked. At irregular intervals man rediscovers one of them, and he is very properly regarded as a human benefactor. Thus, about one hundred years ago, a noted Frenchman named Appert found out that strong sugar

solutions added to fruits increased very materially their keeping qualities, and to-day the housewife adds syrup to her fruit "preserves" as a matter of course. The entire canning industry had its origin in the experiments of Appert. The scientific explanation of the success of the process, however, awaited the development of the science of Bacteriology.

Effect of Desiccation upon Bacteria. Out on the dry, arid plains of the southwestern part of the United States, where the temperature runs high in the middle of the day and especially in summer, meat is preserved by the process known as "jerking." The method is simple. Thin strips of freshly killed meat are hung in the sun to dry. A loss of water takes place rapidly, and within a very few hours the flesh has lost most of its moisture and become quite leathery. A race has taken place between the microbes, which, of course, are present on the meat, ready to decompose it into a mass unfit to eat, and the withdrawal of water from meat and microbe alike by rapid evaporation in the hot, dry air. Evaporation wins, the meat becomes mummified in the hot, dry desert air, and man is assured of a supply of essential food in regions where the iceman is unknown.

Drying, or desiccation, is now practiced on a large scale to preserve food from microbic decomposition. Also, of course, the bulk of the food is materially reduced. Desiccated milk, desiccated fruits and vegetables, desiccated meat, all are imitations of one of the methods of preservation designed by Nature. Meat, milk, vegetables, and fruits cannot be preserved by desiccation in a moist, germ-laden atmosphere. Naturally or artificially hot, germ-free or nearly germ-free air is absolutely essential to the success of the process.

Light without Heat. One of the dreams of the scientist is the production of light without heat. The realization of this highly desirable addition to human achievement is yet to be attained. Nature, however, has been very successful. The pale, greenish light of the firefly and the glowworm, the faint phosphorescence of the mouldering wood in the forest, the gleam of the jellyfish, and the sparkle of the waters of the tropics infested with that minute marine animal known as *Noctiluca* are familiar to those who delight in the beauties of the night. Some kinds of bacteria emit a faint, phosphorescent glow as they develop in suitable cultures in the laboratory, and they give rise to marked luminescence

if they infect fish or other aquatic denizens. One kind of phosphorescent microbe at least appears to be pathogenic for fish. If it gains entrance to the tissues, it soon invades them deeply and eventually permeates the entire body. Such a fish glows all over, and, while death soon intervenes, the interval between invasion and dissolution is one of phosphorescent splendor. It is believed that some of the phosphorescent jellyfish and other aquatic animals may possibly owe their luminescent glow to invasion of their bodies by phosphorescent microbes.

Cultures of phosphorescent bacteria in glass tubes emit sufficient light to make it possible to photograph them by their own luminescence. It is even possible to illuminate a near-by object, as a watch, by their light, and to photograph it. The part luminescent microbes play in Nature is wholly unknown. Their chief interest at present is associated with their ability to produce light without an appreciable liberation of heat.

CHAPTER IV

The physics of bacteria — Relations between surface, volume, and the energy requirements of bacteria — The nutrition of bacteria — Nutritive substances — The microbic Dr. Jekyll and Mr. Hyde — Purification of sewage by bacteria — Food poisoning and food flavors.

The salient features of bacterial activity considered thus far reveal two characteristics not evidenced by larger and more highly organized living things: microbes are minute, and they perform work apparently disproportionate to their size. It should be borne in mind, however, that bacterial multiplication is very rapid. Even within twenty-four hours the descendants of a single microbe may actually number hundreds of millions. Nature not infrequently makes use of a multitude of minute cells to accomplish her purpose where a single cell, having a volume equal to the combined volumes of the smaller units, would fail.

Thus, one of the most important processes essential to life is that of breathing. Man draws about a pint of air into the lungs, and expels a like volume of spent air, eighteen or twenty times each minute. The incoming air brings oxygen, required by the cells of the body to oxidize or burn the carbon nec-

essary for the production of body energy. The outgoing air is rich in the gaseous end product of this combustion, carbon dioxide. The problem imposed upon the breathing or respiratory system is to distribute oxygen to all parts of the body and to collect from all parts of the body the waste carbon dioxide, transport it to the lungs, and there eliminate it. A marvelous mechanism, consisting of the lungs and the blood, represents Nature's solution of this problem. The principle involved is the distribution of the oxygen-containing air over a very large surface in the lungs around which a multitude of very small, rapidly passing oxygen-carriers are paraded, and the simultaneous passage of the carbon dioxide-laden blood over the same area. The lungs, therefore, are the port of embarkation and debarkation. They are saclike structures connected with the outside air by a series of tubes. The business end of the lungs consists of thousands of minute, bulblike cavities, known as "alveoli," each lined with a very delicate membrane, through which blood passes in a thin layer. The *combined surface area* of these alveoli is estimated to be about ninety square yards. The *total volume* of the lungs, however, is less than 400 cubic inches.

The blood contains myriads of tiny, biconcave

discs, known as "red blood corpuscles," or "erythrocytes." Each is seven or eight times the diameter of an average microbe — about one twenty-five hundredth of an inch, in other words. The total number of red blood cells in a man of medium size (170 pounds) would be nearly twenty-seven millions of millions (27×10^{12}, or 27,000,000,000,000). The *combined volumes* of all the red blood corpuscles would scarcely fill a pint measure. The *surface area* of this oxygen-carrying army, on the contrary, would be nearly four thousand square yards (3725 square metres). This is very important. The passage of each red blood corpuscle through the lungs occupies but a very short time. During this time, however, each of these little porters must receive a load of oxygen to carry to air-hungry cells far away in the depths of the body. It is difficult to imagine ninety square yards of surface exposure to the air comfortably tucked away in our lungs and a steady procession of minute oxygen-carriers, with a combined surface of literally hundreds of square yards, constantly receiving this precious life-sustaining cargo wholly without disturbance throughout the span of life. The secret of the success of the oxygen-carrying process lies in the great surface area of the lungs and the great surface area of the

red blood corpuscles — a rapid loading of a multitude of small cargoes.

The relation of surface to volume is best understood from a concrete example. Let a cube ten inches on each edge be considered. From this cube let there be produced smaller cubes, respectively an inch in diameter, and one one-hundredth of an inch in diameter. Inasmuch as the entire material for the ten-inch cube is used in each operation, it is obvious that the total volume of the smaller cubes cannot exceed that of the original cube from which they are to be cut.

The ten-inch cube has a *volume* of $[10 \times 10 \times 10]$ inches, or 1000 cubic inches. The *area* of the *surface* of the cube will be $6 \times [10 \times 10]$ inches, or 600 square inches. Let the cube be cut into one-inch cubes. One thousand smaller cubes will result. The *combined volumes* of the one-inch cubes will be $1000 \times [1 \times 1 \times 1$ inches], or 1000 cubic inches. The *combined surface area* of the one-inch cubes will be $1000 \times 6 \times [1 \times 1$ inches], or 6000 square inches. Now let the ten-inch cube be cut into smaller cubes, each one one-hundredth of an inch on each side: 1,000,000,000 cubes will be obtained, or 1×10^9.

The total volume of 1×10^9 cubes, each 0.01 inch on an edge, will be $1{,}000{,}000{,}000 \times [.01 \times .01 \times .01$

inches], or 1000 cubic inches. The *total surface area* of 1,000,000,000 cubes, each 0.01 on an edge, will be 1,000,000,000 × 6 × [.01 × .01 inches], or 600,000 square inches.

To summarize, the volume of a ten-inch cube, and of the combined volumes of cubes that may be obtained from it by cutting it into one inch, and one one-hundredth of an inch cubes, respectively, are, of course, identical. The surface areas, however, increase with the number of smaller cubes obtained, thus:

	VOLUME	SURFACE AREA
1 10-inch cube	1000 cu. in.	600 sq. in.
1000 1-inch cubes	1000 cu. in.	6000 sq. in.
1,000,000,000 1/100-inch cubes	1000 cu. in.	600,000 sq. in.

The effect of great subdivision, therefore, is to increase the surface area of the fragments very greatly, the combined volume remaining the same. The advantage of a large number of red blood corpuscles depends not upon their combined volume, which is small, but upon the aggregate surface, which is very large. The absorbing surface for oxygen is the desirable feature, and Nature uses a multitude of small surfaces for this purpose.

Bacteria are smaller by far than the cubes measuring one one-hundredth of an inch on each edge. Microbes are even smaller than red blood corpus-

cles. It is very clear that the ratio of their surface to their volume is much greater than that of the smallest cubes thus far discussed. Herein lies their ability to perform an amount of work apparently wholly disproportionate to their size.

It is a general biological principle that the amount of energy required to maintain life varies according to the surface area of the body rather than the weight, or more accurately the volume, of the body in the same species. Thus, a young baby requires proportionately a greater amount of energy to maintain it in a state of well-being than a man. Also, a dog requires proportionately a greater expenditure of energy to keep it in good trim than does an elephant. Bacteria, having a much greater surface in proportion to their volume than man or baby, dog or elephant, require proportionately a much greater expenditure of energy than any of these to maintain themselves in a state of normal activity. This factor of great surface area in relation to bulk or volume, combined with the ability to multiply very rapidly, is the physical basis for the apparently disproportionate activity of microbes in contrast to their smallness, for, in spite of the minuteness of individual bacteria, they bring about changes of appreciable magnitude in their

surroundings. Thus, the rapid decay of a dead animal lying upon the ground in warm weather, the quick souring of milk that is not kept cold, and the unmistakable evidence of change in food left in the family refrigerator after the iceman fails to appear at the proper time, are familiar examples of the rapidity and extent of microbic activity.

Nutrition of Bacteria. Single bacterial cells are invisible to the naked eye. They have as individuals no appeal to human senses. Their important and sole relation to mankind resides, not in what they are, but in what they do. Nevertheless, microbes, man, and all living things exhibit, in common with the inanimate objects which have a function to perform, two definite phases in their life-history. These relate respectively to the structural or formative phase, and the functional, energy, or fuel phase. In point of time, the former precedes the latter. Both are essential. In point of accomplishment, the latter is decisive.

A clear conception of the relation of the structural and energy phases both of animal and inanimate machinery may be attained through a brief presentation of the life-history of a railroad locomotive. A locomotive is built very largely from iron and

steel. When it is structurally complete, it is able to draw a train of cars in virtue of the energy derived from the burning of coal in its fire-box. There are, therefore, two distinct phases discernible in the life-history of the locomotive. The first is the structural phase, which ends, except for the replacement of parts of steel or iron, when the machine leaves the shop. The second is the fuel or energy phase, which is made possible by an available supply of coal. Not only are the structural and energy phases distinguishable in point of time and of materials, but also the amounts of steel and of coal utilized are very unlike. The weight of the steel and iron required to erect a complete locomotive is much less than the tonnage of the coal burned to provide energy for the machine through a period of months or years. Indeed, a locomotive would consume more than its weight in coal in a very short time. Expressed differently, it may be stated that the amount, or weight, of material required to run a steam engine (energy or fuel requirement) exceeds by many times the weight of materials built into its structure (structural requirement).

All known living things resemble the machine built by human hands in that they, too, exhibit both a structural and an energy or functional phase.

There is, to be sure, some overlapping of the phases in the animate machine in point of time. The structural phase, however, is most conspicuous during the period when the living organism is attaining its growth, and the energy or functional phase is clearly discernible when structural maturity is reached, and the life activities are in full operation.

The structural and energy-producing substances available for living things are quite different from those required by machinery, and it is necessary to digress for a moment to call attention to them.

There are five great classes of substances possessing food value. They are:

1. *Proteins and protein derivatives.* These are complex compounds of carbon, hydrogen, and oxygen, which contain nitrogen as their distinguishing element. Nitrogen is the great structural element of the body. It has no energy value, however, although the remainder of the protein molecule furnishes energy upon oxidization. Examples of the proteins are meats, white of egg, gelatin, casein (the basis of cheese), and many vegetable proteins, as gluten.

2. *Carbohydrates.* These are compounds of carbon, hydrogen, and oxygen. They are rich in available energy, but unsuitable for structural require-

ments because they contain no nitrogen. Sugars and starches are examples of the carbohydrates.

3. *Fats.* Fats, like carbohydrates, are complex combinations of carbon, hydrogen, and oxygen. They contain no nitrogen. Like the carbohydrates, they yield energy when they are consumed (or burned) in the body, but they also give up relatively much heat at the same time. Familiar examples are fats of various kinds (vegetable and animal), oils, butter, oleomargarine.

4. *Mineral salts.* Mineral salts furnish no energy of themselves. They play an important part, however, in the vital economy, partly by regulating bodily conditions, partly as important constituents of skeletal structures. Bones contain much lime and phosphorus. All tissues of the body contain some minerals. Sodium salts are particularly craved by the herbivora. Potassium salts are important for plants. Milk is rich in mineral substances required for growth.

5. *Water.* About eighty-five per cent of the tissues of animals and the substance of bacteria is water.

To return to the structural and energy requirements of bacteria, animals, and man: nitrogen is

the significant structural element of life, precisely as iron is the essential structural element for the locomotive. A locomotive cannot burn iron for fuel; neither can bacteria, animals, nor man burn nitrogen for energy. Both the steam engine and the living machine burn or oxidize carbon for energy. Just as the non-combustible impurity of coal — slag — is not burned in the fire-box of the engine, but remains unconsumed as ash, which must be removed, so the non-combustible nitrogen or organic energy-containing substance, as proteins and their derivatives, are not oxidized in the tissues of animals or man, and must be eliminated. From the viewpoint of energy, therefore, coal which leaves little or no ash, and organic compounds which contain no nitrogen, are theoretically the most suitable for both the inanimate and the animate machine.

Bacteria likewise require a supply of utilizable nitrogenous substances, as proteins or protein derivatives, together with organic compounds of carbon, hydrogen, and oxygen, mineral salts, particularly phosphorus, water, and a suitable temperature for their growth and development. There are many kinds of bacteria, some performing a multitude of useful transformations in the economy of Nature, some living as parasites upon man or animals, and in

some instances even inciting disease in plants, animals, or man. Their requirements for structure are nearly as varied as their functions. Nearly all microbes, however, can utilize one or more kinds of sugar for energy. This is very important.

Soil bacteria may derive their structural essentials from relatively simple nitrogenous compounds, as those resulting from decaying plant or animal remains. Others multiply best when quite highly organized nitrogen-containing substances — as peptones or other partially digested animal tissues — are available. The proteins of milk are well adapted to the needs of many parasitic organisms. Some microbes, however, are exquisitely fastidious in their nitrogen requirement, and develop normally only when proteins as highly complex and specific as the unaltered, fresh tissues of the human or animal body are provided for them.

Experience has shown that a very satisfactory medium for the cultivation of a majority of bacteria may be prepared from a meat infusion (beef tea), preferably reinforced with peptone or other partially digested material belonging to that great class of nitrogenous organic compounds known as "proteins." A nutrient medium prepared thus with meat extractives and peptone is known as "plain,

nutrient broth," or, more simply, as "plain broth." This is also the *basis* for a great number of special media used in the study of bacteria. From what has gone before, it is quite obvious that the protein constituents of plain broth provide both the structural and energy needs of bacteria. This means that the energy moiety of the microbe's growth must be obtained from the nitrogenous constituents of the medium. Prior to the oxidization of the protein molecule for the fuel requirements of the organisms, the non-combustible nitrogen must be removed. This, theoretically at least, requires an expenditure of energy.

Sugars, which contain readily available carbon, hydrogen, and oxygen, contain no nitrogen; they are frequently added to plain broth to supply a readily utilizable source of energy. In such a medium the bacteria must, of course, use the nitrogenous substances for their structure. They have a choice, as it were, between the protein and carbohydrate for their energy. It is not a matter of indifference in the performance of the functions of bacteria which is used. A few illustrations will be illuminating.

One of the formidable bacteria which incites disease in man is that microbe found in every case of

true diphtheria, and, therefore, appropriately called the diphtheria bacillus. This microbe may be cultivated outside the human body in the plain broth medium. Among the soluble substances produced by the diphtheria bacillus as a result of its growth in plain broth is a very powerful poison (diphtheria toxin) which may be obtained free from all microbes by the simple expedient of passing the culture through stone filters. The pores of the filter restrain the bacteria, but permit of the free passage of the soluble products of its growth. A very small amount of this filtered fluid, even as little as a fiftieth of a drop, will contain enough of this soluble poison, or toxin, to kill a medium-sized guinea pig within four days with all the essential symptoms of diphtheria intoxication. This toxin is more potent than the most powerful drugs. Consider now the effect of adding a non-nitrogenous source of energy, as that simple sugar known as "glucose," to the plain broth medium. As before, the meat extractives and peptone must furnish the structural needs of the diphtheria bacilli, but the microbes have a choice between the proteins and the glucose for their energy requirements. Glucose is more readily utilized for energy than peptone, and the microbes so use it in the glucose broth. The result of this

utilization of glucose in place of protein as a source of energy for the diphtheria bacillus is striking, indeed. The soluble products are no longer poisonous; even in large amounts, they are without noteworthy effect when injected into guinea pigs. The principal substance formed is lactic acid, the chemical basis of buttermilk.

The story of Dr. Jekyll and Mr. Hyde, that strange and imaginary conception of a dual human personality, has its actual realization, and far more striking and realistic, in this simple experiment upon the energy requirements of the diphtheria bacillus. In plain broth the microbe produces a potent toxin which confers on the bacillus its formidableness in producing disease. The simple addition of glucose as a readily utilizable source of energy for the organism so changes the nature of its growth products that they are not only no longer toxic — they are potentially possessed of food value. They are actually the chemical equivalent of buttermilk.

This striking relationship between the nature of the substances used by the diphtheria bacilli for their energy and the character of the products they form is by no means an isolated occurrence — it is but a single instance illustrative of a very general

principle of bacterial nutrition encountered in many fields of microbic activity.

PURIFICATION OF SEWAGE BY BACTERIA. One of the important adaptations of bacterial action to human needs is the purification of sewage and of water in so-called sand filters. The essential feature of a sand filter, or biological filter, is a continuous layer of bacteria, resting upon the surface of a bed of sand. The sewage or water to be purified — that is, to be rid of the organic waste of man which it contains — passes downward through this living carpet of microbes, and is freed during its passage from its undesirable substances. These organic substances are actually digested by the microbic population of the filter, and transformed thereby into simple, inoffensive residues suitable for plant food. The sewage or water thereupon ceases to be a nuisance. The organic constituents of sewage and polluted water are for the most part nitrogen-containing. The bacteria resident in the filter use these substances for their energy as well as for their structure and in so doing eliminate the nitrogen from its organic combination and oxidize the carbon residue. It was found, many years ago, that the addition of sugar to sewage or impure water prior to its diges-

tion in the sand filter arrests the process of purification to a very considerable degree, and thereby destroys, temporarily, the usefulness of the process. As soon as the sugar is eliminated, the process of purification again becomes effective. The explanation seems simple: the filter microbes can derive their energy more readily from the non-nitrogenous sugar than from the nitrogenous wastes of man. In consequence, the nitrogenous constituents are avoided by the microbes, aside from the relatively insignificant amount required for their structural needs, and the prime object of the filter — the degradation of these nitrogenous wastes to simpler, inoffensive combinations — is thereby frustrated. Removing the sugar forces the bacteria to work upon the nitrogen impurities for their energy.

Another instance of this sparing action of sugar for nitrogenous substances occurred several years ago in connection with the egg industry. Eggs which are rather old, or slightly damaged by handling, but otherwise intact, are salvaged and sold to bakers. The eggs are removed from their shells, thrown into cans, together with about ten per cent of their weight of sugar, frozen, and in this state disposed of for wholesale culinary purposes. They appear to be quite satisfactory for this purpose.

Not infrequently, however, the bacterial content of these frozen eggs may be quite considerable. Samples of some of these frozen eggs were seized by the Government, examined bacterially, and condemned, to use the language of the Pure Food and Drugs Act, as being "filthy, rotten, and decomposed," because of their high content of bacteria. It is, of course, a well-authenticated fact that eggs may under certain conditions deserve this scathing indictment, as every one who has lived upon a farm very definitely understands. The eggs under discussion, however, in spite of their large microbic population, were found to be quite as free from noxious or disagreeable odors as the most authentic of freshly laid eggs. Chemical examination failed to reveal any evidence of decomposition. Cakes made with these frozen eggs, and from eggs of known vintage, were prepared in the courtroom and passed to the jurors, who were unable to distinguish between the two kinds. Taste, smell, and sight alike were incapable of perceiving any difference. It so happened that the microbes specifically incriminated by their presence as being the cause of the seizure were those which could use the sugar for their energy. Because of this fact, it would seem that foul products resulting from the microbic decomposition of the proteins

of the eggs would hardly be expected to develop in such a mixture. It is unnecessary to add that the case against the eggs was unsuccessful. Instances of this "sparing action" which sugars exhibit, shielding nitrogenous substances from degradation by bacteria for energy, might be multiplied many times, but the principle involved is the same.

Food Poisoning, Food Flavors. Other examples of the action of microbes upon proteins or protein derivatives, however, deserve mention as illustrative of the remarkable versatility of various types of germs upon the same general kind of substance.

At one end of the series is that group commonly referred to as "food poisoning," a term which is used somewhat loosely to include several distinct types of bacterial poisons. At the other end of the series is that group of changes, also of microbic causation, which is deemed highly desirable by epicures. The "highness" of game, many of the flavors of cheeses, and other partial decompositions of nitrogenous food products are illustrative. Food poisoning deserves a word of comment. There are several kinds. The most formidable types are recognizable as the specific action of well-known bac-

teria. Botulism, a severe or fatal poisoning induced by eating foods containing the toxin of *Bacillus botulinus*, is one of these. The first epidemic to be carefully worked out occurred among the members of the church choir in Ellenzelles, a little town in Belgium. The circumstances were dramatic. After the evening practice was over, the members of the church choir sat down to refreshments. The principal dish was some pork sausage, which unfortunately had been imperfectly cured. Several who partook of this died. A celebrated Belgian bacteriologist investigated the outbreak, which presented some characteristics new to science, and added a new, or rather a hitherto unrecognized, microbe to the list of man's enemies. Within recent years, spinach, beans, asparagus, and ripe olives, which are preserved in hermetically sealed cans, have been found to be infected with *Bacillus botulinus*. Several deaths have resulted and much apprehension has been aroused in the public mind concerning the dangers inherent in these particular foods. Fortunately, very little additional treatment, now quite fully understood, suffices to remove all danger from this source.

Another type of food poisoning associated with beef or pork, containing members of that group of

microbes known as "paratyphoid" bacilli, has upon occasion been the source of limited epidemics of considerable severity. Careful slaughtering and handling of meats, however, reduces the danger from this source to a minimum. It should be stated that thorough cooking of food destroys both the soluble poison of *Bacillus botulinus*, and the poison and the growth of paratyphoid bacilli.

In the fall, when the thrifty housewife or landlady, tormented by the cost of ice, relies upon cool weather to preserve her food, outbreaks of so-called food poisoning are quite common. These usually follow an unexpected warm day and night, when the ice-box is depleted of ice and none can be had for a period of time. Meats and other foods, exposed to the relatively warm temperature of the iceless ice-box, undergo decomposition by the activity of their resident microbic population. As a rule, true toxins are not formed, but the partially decomposed nitrogenous substances may be, and frequently are, sufficiently revolting to the digestive system to upset its function for several days. Fortunately, the results are rarely fatal.

A distinction can scarcely be made, chemically speaking, between these mild, digestive disturbances resulting from the decomposition of foods in

the iceless ice-box and that epicurean state of decomposition spoken of in association with game as "high." The height of "high" which an untrained digestive system can tolerate is varied; some can stand more and some less. The very "high" game would in all probability be nearly as intolerable to delicate digestions as the over-ripened meat of the warm ice-box. On the other hand, the changes which are concerned in the ripening of game are relative, in that at least the earlier stages are really required to make the meat palatable. The same is true of the various kinds of cheeses; there is no uniformity of taste in determining the extent to which microbic decomposition must attain before a satisfactory flavor is developed. There should be no unnecessary alarm concerning the possible dangers of microbic contamination of foods put up under Government supervision; cleanliness, chilling, and conscience in handling by retailers and in the home are ample safeguards against accident.

The microbes of the meat-poisoning group, and, so far as they are known, that group which plays some part in the ripening of meat and cheeses, and in the development of desirable flavors in protein foods in general, the diphtheria bacillus, the bacteria of sand filters, and in fact the great majority of

all known bacteria, exhibit a very definite relationship between the nature of the organic substance they utilize for energy and the character of the significant products they form, as a result of their energy transformations.

The diphtheria bacillus grown in "plain broth" distinguishes itself from all other microbes by the soluble toxin it forms. Similarly, the typhoid, cholera, dysentery, glanders, and meningitis germs, paratyphoid and other members of the food-poisoning group, and, indeed, the majority of bacteria progressively pathogenic for man, produce harmful products, each specific for its kind, from the common nutrient medium, "plain broth." Each microbe, in other words, elaborates its specific poison from the same initial ingredients. This has its counterpart in the human body, where again diphtheria, cholera, dysentery, typhoid, and the rest of the pathogenic group, induce changes in the same medium, the tissues of man, each specific for its kind.

On the other hand, the addition of sugar that can be used as a source of energy to plain broth alters the nature of the products formed by the several microbes very distinctly and characteristically. The presence of glucose in cultures of diphtheria, typhoid, paratyphoid, dysentery, cholera, and

other formidable incitants of human infections, reduces all of these bacteria to one general chemical type — lactic acid producers. All of these microbes, it seems, exhibit this dual rôle of Dr. Jekyll and Mr. Hyde, with its important feature that the benign (lactic acid) aspect is the same for all of these bacteria. They all form lactic acid as their chief significant product when they utilize sugar for their energy. It is quite obvious, therefore, that the formidableness of these microbes is associated with their utilization of protein for energy. Also, their specificity — that is to say, their characteristic and distinctive products of growth — is dependent upon protein utilized for energy. Diphtheria toxin is only produced, so far as is known, when the diphtheria bacillus uses the protein of cultural media, or the protein of human or animal tissues for energy. When glucose is available as a source of energy, the microbe becomes potentially a sour milk, or lactic acid, producer. In the same manner, the nitrifying bacteria of sand filters, the microbes of the frozen eggs, and a multitude of bacteria in many fields of microbic activity, are microscopic Dr. Jekylls and Mr. Hydes. Energy manifestations are as important among bacteria as they are among the children of man. "By their fruits, ye shall know them."

CHAPTER V

The nutrition of bacteria, continued — Toxin and antitoxin — Vital specificity of bacteria — Microbes and sugars — Structure of sugars — Polarized light — The polarimeter — Optical properties of sugars — Bacteria as sugar chemists.

THE principles established in the last chapter are of sufficient importance to warrant their presentation once again in concise form.

1. Bacteria, like all living things, show two very distinct phases in their life-history: the *structural phase*, during which the microbe grows to its full size as a mature organism, and the *energy phase*, in which the microbe enters upon and performs its life-work.

2. The respective magnitudes of the structural and energy phases are wholly unlike. The structural requirement is little, indeed. It will be recalled that five hundred million average-sized, fully mature bacteria weigh about a milligram. The energy phase, on the other hand, which is the visible manifestation of microbic activity, is relatively very great.

3. Nitrogen-containing substances are an absolute requirement for the structural phase of bacterial development. Properly combined nitrogen is

an essential for the formation of bacteria, precisely as iron is a necessity for the building of a locomotive.

4. The oxidization of carbon is the basis for bacterial energy, precisely as the burning of coal (which is also an oxidization of carbon) is the basis for the energy of the locomotive. In other words, bacteria cannot exist without nitrogen and cannot function without carbon, any more than a locomotive can exist without iron or function without coal.

Bacteria are, or may be, very fastidious in their choice of nitrogen-containing substances for structure, and equally so in their choice of carbon-containing substances for energy. A broad and practically untouched field of Bacteriology awaits the development of methods of precision in which this fastidiousness of microbes is made use of by employing them as living chemical reagents.

Toxin and Antitoxin. One aspect of this utilization of bacteria as diagnostic reagents has received much attention. This is in association with medicine. Here empirical procedures have been discovered in which specific invading microbes have been used in the diagnosis of disease. The underlying principle involved depends upon two impor-

tant facts: First, that each invading microbe produces its characteristic and unique poison which stimulates the host — the invaded organism — to react by the production of a specific counter-poison or a specific microbicidal substance. Secondly, these counter-poisons or microbicidal substances, produced as a result of invasion by a specific microbe, are effective only against that microbe whose growth in the body called them into being. Thus, the diphtheria bacillus produces its specific and characteristic soluble poison, or toxin, either from the ingredients of plain broth in cultures outside the human body or within the tissues of man. This poison, injected into animals in gradually increasing amounts, or produced gradually in the tissues of man, stimulates the living cells of the body to form a soluble substance — an antitoxin — which will neutralize, and therefore render inert, the specific poisonous product resulting from the utilization of protein by the diphtheria bacillus. The poison-neutralizing or antitoxic serum specific for the diphtheria toxin will be wholly without effect upon the toxin of the lockjaw bacillus, or, indeed, upon the toxin of any microbe other than that of the diphtheria bacillus. It should be emphasized that the *living* microbe produces its specific poison or

toxin and that the *living* man or horse elaborates within its body the specific antitoxin.

VITAL SPECIFICITY. This high degree of specificity is characteristic of all living things. Not only does the diphtheria bacillus elaborate its highly specialized poison, different from all other known chemical compounds; but also each plant and animal and man exhibits specificity. The thousands of varieties and kinds of plants, each after its own kind, utilize the same ingredients, oxides of nitrogen, carbon, and hydrogen, drawn from the soil, and, so far as known, the same chlorophyll in their leaves, and the same energy of the sun's rays. From these common elements, drawn from the soil and the sun, each plant moulds its own architectural design, through the activity of its chlorophyll. It never makes a mistake. A clover plant does not, and apparently cannot, alter its plan and grow in the image of a buttercup. A cow, feeding upon the clover or the buttercup, cannot produce offspring resembling the camel. It does seem to be possible to cross related plants and animals and create hybrids, but this is quite apart from the present discussion.

Bacteria, plants, animals, and man, therefore, exhibit remarkable specificity. From the same in-

gredients each produces chemical combinations of sufficient difference to confer upon the species its identity. In general, the nitrogen-containing substance of plants, derived from the nitrogen oxides of the soil as a common starting-point, is as distinctive chemically as is the architectural design of a locomotive, a watch, or a battleship, all of which are constructed of iron as the basic element.

The complex proteins, carbohydrates, and fats, which are left as lifeless residues when plants or animals die, are broken up by bacteria in the soil, and the valuable elements therein contained are restored to the cycle of life through this activity of the living earth. Many kinds of microbes participate in this process. Some remarkable instances of the versatility and the specificity of bacterial discrimination between closely related substances are involved in the processes of this cycle of the elements. In some instances, at least, microbes will distinguish very sharply between two substances which may elude the combined powers of man and instruments of precision. A few illustrations will make this clear.

Microbes and Sugars. It was stated in the discussion of the organic substances used respectively

for structure and for energy by animals and bacteria which possess no chlorophyll, and consequently depend upon these substances elaborated by the green plants, that proteins or protein derivatives (nitrogenous substances) were an absolute requirement for all living things that are parasitic upon plants. The energy requirement of these living things, however, can be derived either from members of the protein group or from the carbohydrate group.

Microbes utilize carbohydrates in preference to protein for energy, if both are available in the same medium. It will be recalled that the usual product resulting from the oxidization of carbohydrate for energy is acid in character. Generally speaking, the utilization of protein for energy results in the formation of ammonia and other substances which are alkaline. Enough acid or alkali may be formed in a short time, due to the rapidity of growth of microbes and their relatively large energy requirement, to permit of a simple method of recognition between the two alternatives. A piece of litmus paper will turn red if it is dipped in a solution containing even a small amount of acid; it will become blue if alkali is present. Starting with a solution neutral to litmus in its reaction, microbic utilization of protein

for energy, or of carbohydrate for energy, is readily determinable with this simple litmus indicator.

Herein lies the basis for a number of important chemical applications of the versatility and specificity of microbic action.

The carbohydrates, comprising sugars, starches, and some additional combinations of carbon, hydrogen, and oxygen, are an extremely interesting and important group to the chemist. They are the cheapest and most economical sources of energy for mankind. Also, the carbohydrates are among the most remarkable substances produced by the synthetic activity of plants. Cane-sugar, starches, grape-sugar, and many others are familiar names of important foods. There are literally hundreds of sugars, glucosides, and starches however, strikingly alike in their empirical composition and quite unlike in their various reactions to chemical reagents.

Structure of Sugars. One of the simplest sugars and the most common is glucose; it is also called grape-sugar, dextrose, and several other names. It is found in the juices of many plants; it is the sugar found in the blood-stream of man and animals, and it is the most widely distributed of all the sugars in nature. Chemically, glucose is com-

posed of six carbon atoms, twelve hydrogen atoms, and six oxygen atoms. Its empirical formula, as the chemist says, is $C_6H_{12}O_6$. It should be stated that the initial letters of the elements are generally used to designate them — a sort of chemical shorthand, as it were. Experience has shown that glucose cannot be represented as a chemical entity by the simple formula $C_6H_{12}O_6$, because there are at least sixteen possible distinct chemical compounds containing the same number of atoms of these three elements. Four of these, glucose, fructose (lævulose), mannose, and galactose, are represented in the following diagram:

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                        H
                        |
(1)   H-C=O         H-C-OH      H-C=O        H-C=O
        |             |           |            |
(2)   H-C-OH          C=O      HO-C-H       HO-C-H
        |             |           |            |
(3)  HO-C-H        HO-C-H      HO-C-H        H-C-OH
        |             |           |            |
(4)   H-C-OH        H-C-OH      H-C-OH       H-C-OH
        |             |           |            |
(5)   H-C-OH        H-C-OH      H-C-OH      HO-C-H
        |             |           |            |
(6)   H-C-OH        H-C-OH      H-C-OH       H-C=O
        |             |           |            |
        H             H           H            H
     Glucose       Fructose     Mannose     Galactose
                  (Lævulose)
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Polarized Light. Each of these four of the possible sixteen sugars, containing six carbon, twelve

hydrogen, and six oxygen atoms, possesses the ability of rotating the plane of polarized light a different amount. By polarized light is meant a beam of light whose waves move in the same plane of space. Light from the sun, or any ordinary terrestrial source, is made up of waves which vibrate in different planes of space. In this respect it is different from a wave of the ocean. The waves that break upon the beach have their crests and troughs parallel. It is possible to make the rays of light parallel if all vibrations except those in one plane are eliminated. Certain transparent crystals, as Iceland spar, are so constituted that waves of light, except those that will pass in one definite direction parallel with the axis of the crystal, are reflected to one side. The light which comes through the crystal has been rectified, as it were, and vibrates in a single plane, much like ocean waves. Scientists have discovered substances which will deflect a beam of rectified or polarized light to one side or the other, just as a breakwater placed at an angle to the incoming waves will deflect the breakers. The direction and extent of the deflection depends upon the amount of the substance dissolved in water, and the length of the column of it through which the beam of polarized light is passed.

THE POLARIMETER. An instrument of precision, known as a "polarimeter," is used to measure the extent of the deviation of the beam of polarized light, as one or another so-called "optically active substance" is placed in its path. Two crystals of Iceland spar are required; one to rectify or polarize the incoming light, the other, movable in an arc, to receive the light after it has been deflected by passing through the optically active substance. The angle between the direction of the incoming light at one end of the polariscope and that after it has been deviated in the solution of optically active substance is spoken of as the optical or angular rotation of the substance under discussion.

It follows that a definite weight of a pure optically active substance dissolved in pure water and placed in a tube of appropriate length will always deviate the plane of polarized light in the same amount, other conditions being the same, because the property of rotating the plane of polarization is an inherent characteristic of the structure of the substance itself. Many kinds of substances rotate the plane of polarization. In no group of compounds, however, is this more clearly shown than in those containing carbon.

Optical Properties of Sugars. Returning to glucose, fructose, mannose, and galactose: each of these substances has a specific rotation. Glucose turns the plane of polarization 52.5° to the right; fructose, 93.0° to the left; mannose, 14.0° to the right; and galactose, 80.4° to the right. It should be remembered that the optical rotation of a solution depends upon the amount of dissolved substance as well as the length of the solution through which the measurement is made.

A very small amount of glucose, for example, existing as an impurity of mannose, would escape detection in the polariscope. Indeed, it would be beyond the ken of even the most skilled chemist, using the most refined apparatus, to detect and identify a tenth of one per cent of glucose existing as an impurity in mannose. The two sugars are so nearly alike in all their properties that they are distinguishable only in relatively large amounts.

Bacteria as Sugar Chemists. That same wonderful definiteness, however, that caused plants, each after its own kind, to fashion these closely allied, but entirely distinct, chemical substances known as sugars, has its counterpart in a corresponding relationship between the arrangement of the elements

of these sugars in space and their utilizability by the living substance of bacteria for their energy requirements.

Looking back to the diagram on page 76, it will be seen that glucose and mannose differ solely in the arrangement of hydrogen and oxygen around the carbon atom in line two. In glucose the order is HCOH; in mannose it is the reverse, the mirror image of this arrangement, HOCH. This seems a small distinction — a mere reversal of two groups around one of the six carbon atoms — and yet it suffices to make one of these sugars a source of energy for certain bacteria, and the other a wholly inert, useless foreign body. Like differences are found, microbially speaking, between all the sugars of this series. Every known organism, utilizing any of the series, will utilize glucose for its energy. If a plain broth medium is made and reinforced, some with glucose, some with fructose, or mannose, or galactose, the microbe may be tested for its fermenting powers. If, under the conditions of experiment, acid develops as the result of the growth of the microbe in each of the four sugars, then the living substance of the microbe is able to derive its energy from each of the four different configurations indicated. Some microbes do this. Others are more

fastidious, and therefore less adaptable in their energy requirements. They may utilize one or two sugars of this list besides glucose. Which ones may be determined readily by the acid formation already mentioned.

It is clear that there are two possible uses to be made of these facts: the first is an identification of specific sugars, using microbes of known sugar-fermenting characteristics, as analysts of proved integrity; the other, using known sugars, to identify unknown microbes. The mutual relations are shown diagramatically:

SUGAR

ORGANISM:	GLUCOSE	FRUCTOSE	MANNOSE	GALACTOSE
A	Acid	Alkali	Alkali	Alkali
B	Acid	Acid	Alkali	Alkali
C	Acid	Acid	Acid	Alkali
D	Acid	Acid	Acid	Acid
E	Acid	Alkali	Acid	Alkali
F	Acid	Alkali	Alkali	Acid

Any microbe or any sugar in this list may be identified as above indicated.

An important and interesting question immediately arises — How delicate is this reaction? How small an amount of utilizable sugar will give suffi-

cient chemical evidence of its presence to be diagnostic? Available information indicates that as small an amount of sugar as 0.0025, or possibly 0.001 of one per cent by weight of glucose in a suitable culture medium, may be detected by the use of bacteria as chemical reagents. This is by far a smaller amount of glucose than can be determined by the most refined chemical reagents.

Many adaptations of this relationship between the structure and arrangement of sugars and the ability of bacteria to distinguish between these several arrangements suggest themselves. Thus, in addition to the relatively simple sugars of the glucose series containing but six carbon atoms, there is a multitude of sugars and starches containing from two to thirty or more carbon atoms, each presumably as definite in its structure and composition as the sixteen possible kinds of sugars with six carbon atoms. Usually these compound sugars and starches are composed of two or more simple sugars, combined through the abstraction of one or more HOH groups. Such combined sugars may frequently be reconverted into their simpler components by the action of heat, dilute acids, and other reagents. Bacteria may be used to identify those components with great advantage. A list of a few of the better-

known compound sugars, together with their component simple sugars, will indicate the nature of this combination.

Name	Composed of
Maltose (Malt-sugar)	Glucose + glucose
Lactose (Milk-sugar)	Glucose + galactose
Saccharose (Cane-sugar)	Glucose + lævulose

The optical, physical, and chemical properties of these compound sugars are quite unlike those of their constituent simple sugars; their properties, in other words, are not the algebraic sum of their elements. Thus, the optical rotation of maltose is $+140.6°$, lactose $+52.5°$, and saccharose $+66.5°$; these figures are not the sums of the rotations of their elementary sugars.

Similarly, the biological properties of the compound sugars are not algebraic sums of their individual components. Microbe A, which ferments glucose, may not ferment glucose + glucose = maltose. Microbe B, which utilizes glucose and fructose with equal readiness for energy, will not necessarily be able to derive energy from glucose +

fructose = saccharose. Indeed, at least two sugars composed of glucose + fructose minus HOH are known, each wholly distinct from its fellow. It makes much difference whether the formula is written glucose + fructose, or fructose + glucose. Space does not permit of an explanation, but some idea of its possibilities is readily ascertained when one reflects on the sixteen possible sugars of the formula $C_6H_{12}O_6$.

Precisely as specific microbes utilize one or more than one of the members of the sugar group having six carbon atoms, glucose, lævulose, mannose, etc., and this fact may be taken advantage of in identifying these sugars, so the fragments of the compound sugars may be identified by microbic chemical reagents. A simple illustration will be explanatory. Saccharose is compounded from a molecule of glucose and a molecule of lævulose. Upon careful cleavage of saccharose, there remain as fragments glucose and lævulose. These sugars may be identified by the bacteria shown in the table on page 81. Even more complex sugars may thus be fractionated and identified by the microbic chemist.

Many problems of interest in the chemistry of life are unsolved, and some of these, at least, may be susceptible to bacterio-chemical procedures.

For example, the urine may contain one of three or four sugars in small amounts in certain types of disease or in cases of unusual physiological activity. Frequently, the amounts are small, and it is difficult to distinguish between sugars that may be of grave import — as an excess of glucose — and those, as lactose, which may be of much less significance. It seems quite possible to utilize microbes for the purpose of distinguishing between these different conditions.

Finally, bacteria will be found to be useful in detecting and even measuring small amounts of impurities in sugars that are to be used as standards in measurements of precision. Many of these impurities are known, but no satisfactory chemical methods are available at present for identifying minute quantities. The uncanny accuracy of bacteria as appraisers of optical antipodes will in time place them in the list of indispensable reagents for the identification and standardization of the great group of carbohydrates. The development of the vast possibilities of bacteria as chemical reagents is one of the fascinating fields of investigation for the future.

CHAPTER VI

The life cycle of the earth — The living earth and the rotation of the elements in the life cycle — The nitrogen cycle: nitrogen-fixation by the leguminous plants and the nodule bacteria — The clover-bacteria complex — The sulphur and iron and phosphorus cycles.

THE chronicle of the origin of life upon Mother Earth, that most wonderful episode in her history, is hidden in the vast record of the ages. All evidence of the character and extent of primeval life, and all traces even of its ephemeral existence, are lost in the abyss of time.

Life, as revealed from its earliest authentic vestiges to the wealth of present-day manifestations, depends for its maintenance and perpetuation upon the continuity of a cycle. The driving force of the life cycle is the *radiant energy* of the sun. The transformer of the radiant energy to life energy is the green coloring matter of the vegetable kingdom, the chlorophyll. Chlorophyll is Nature's master chemist, and Nature's master laboratories are in the leaves of the green plants. There the simple compounds of nitrogen (nitrates), carbon (carbon dioxide[1]), and water and salts brought to

[1] Much carbon dioxide is brought to the leaves in the air, also.

the roots of the plant in solution in the subsoil water and carried to the leaves by the plant sap, are woven into those complex, intricate combinations of nitrogen, carbon, hydrogen, and oxygen which compose the several tissues of the living plant. Man, with all his laboratories and reagents and sources of energy and skill, has thus far been unable to transform nitrates and carbon dioxide into living substance. Directional stimulation seems to be imparted to the chlorophyll by the plant itself, which results in a reproduction of its traditional architecture and chemical specificity. This is the constructive or anabolic phase of the cycle of life upon our earth.

Herbivorous animals feed upon the green plants; carnivorous animals prey upon the herbivorous animals, and omnivorous animals and man subsist upon both the herbivorous animals and plants.

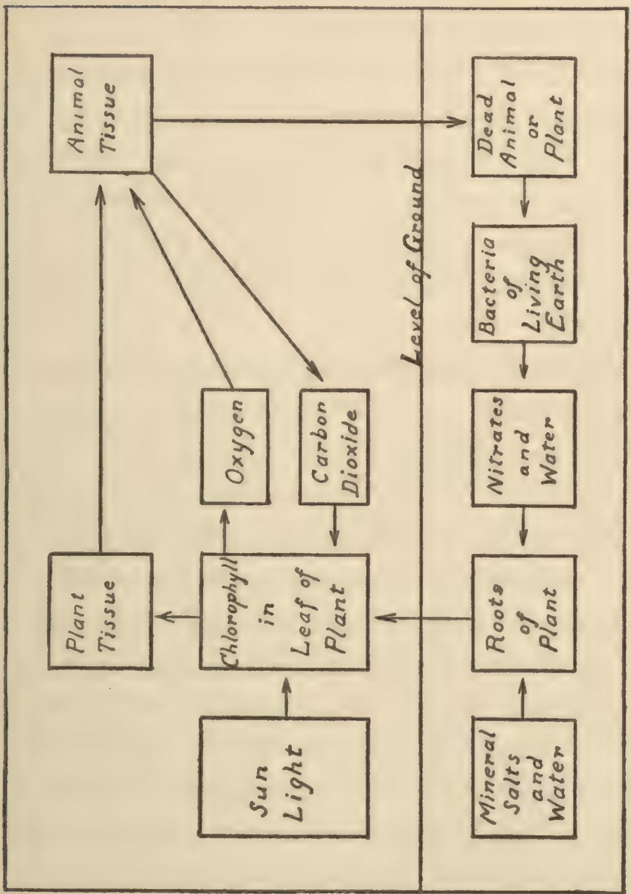
Valuable elements are built into the substance of plants and animals; elements of which the available supply for the upkeep of the cycle of life is limited. These elements are purposefully active so long as the plant or animal performs its functions. Upon the death of the plant or animal, these elements become an inactive, and, because of their high state of organization, an unavailable residuum locked up

in the remains of the organism. But they are very valuable and essential in their original, mineralized form for resynthesis and reparticipation in the cycle of life.

It is the function of bacteria to demolish these compounds, and to restore their valuable elements in utilizable form to the plant kingdom. Without the participation of microbes in this cycle of the essential elements, life would inevitably cease upon this planet.

Notwithstanding the rapidity and completeness with which bacteria degrade the tissues of dead animals and plants, and the products of their vital activities, to nitrates and carbonates and other simple combinations suited for resynthesis by plants, there are losses which diminish the available supply of plant food to a point which would create a deficit, and therefore a restriction of growth, were it not for other agencies which replenish the vital capital.

It is not difficult to realize that the cold of winter retards microbic activity very materially, and thus reduces for the time, at least, the transformation of organic residua to oxidized derivatives thereof almost to the vanishing point. It is unavoidable, also, that escape of valuable compounds dissolved



THE CYCLE OF LIFE

in the subsoil water will occur both in winter and in summer. These are carried to the ocean eventually, and therefore lost for the most part to the land plants. Hundreds and hundreds of tons of nitrogen in the aggregate are carried to the ocean each year. The old agricultural practice of burying fish in freshly seeded soil was an empirical but satisfactory attempt to provide essential elements for plant growth from the great nitrogen repository of the sea.

Despite the obvious losses of nitrogen, however, the cycle of life upon the land goes on. One of the greatest contributions of the microbe to the perpetuation of life is the restoration of utilizable nitrogen to the plant kingdom through the fixation of the uncombined nitrogen gas of the atmosphere. The atmosphere of the earth is an aerial ocean containing about seventy-nine per cent of nitrogen, about twenty per cent of oxygen, and one per cent of other gaseous substances. The nitrogen of the atmosphere is a free, uncombined gas, and in this elemental form it is very inert. It does not combine readily with other elements, and in the gaseous, uncombined state it is useless for the needs of living things. When, however, it is united with oxygen or hydrogen, it is a very valuable — an absolutely

essential — factor for the structural development of plants.

The lightning flash, in virtue of its energy, can and does force small amounts of nitrogen and oxygen to become associated chemically, forming a new substance, an oxide of nitrogen. The oxide of nitrogen, unlike its gaseous components, is a solid and soluble in water. In this form, nitrogen is a most important plant structural compound. Unfortunately, the amount of nitrogen oxides formed by the discharge of lightning is small in amount and irregular in its occurrence. The losses of nitrogen oxides to the ocean must be hundreds of thousands of times greater than the addition of oxides of nitrogen to the soil from the electric discharge. It is worthy of note that man is utilizing the electric energy derived from a conversion of the power of the waterfall to produce nitrogenous plant food from the great nitrogen store of the atmosphere.

Farmers have long known that the continuous planting of crops upon a field or garden leads within a few years to a marked reduction in the return. In virgin soils, as the prairies of the United States in the pioneer days, several years would be required before the ill effects of continuous cropping would be apparent; in well-established agri-

cultural districts each successive yield would show some diminution. Old civilizations, as western Europe and especially China, have learned through years, even centuries, of experience that the soil is not a bank of unlimited credit from which useful plant products may be withdrawn without restoring some of the loss in elemental substances. Of these substances, potash, phosphorus, and available nitrogen are the most important. Potash and phosphorus are largely chemical problems, and, as such, have no immediate part in this discussion. Nitrogen, on the other hand, has been in the past, and still is to a very large degree, inseparably associated with the cycle of life.

Long years of experience have shown that well-rotted manure is an excellent nitrogen fertilizer; its use far antedates the knowledge of the chemical processes involved in its use. Also, the advantage of permitting the soil to be unplanted for a year or two — to be fallow, as the agriculturists call it — is well known. Such resting soils regain somewhat their original fertility.

The most striking example of that keenness of observation for which the farmer is justly noted, however, is the discovery that certain crops, as clover, may be grown upon nitrogen impoverished

soil, ploughed under, and thereby actually increase the nitrogen fertility of the land. The scientific basis for this important discovery was not evident for many decades. It awaited the rise of a new science, Bacteriology, for its elucidation, as well as knowledge of the microbiology of the soil, and a careful botanical study of the kinds of plants which would thus enrich the land with nitrogen. The botanists finally discovered that a particular group of plants, known as the *Leguminosæ*, of which clover is a member, is especially concerned in the nitrogen-fixation process. Upon the roots of these plants, small but clearly discernible nodules may be found which possess peculiar properties.

Within these nodules are microscopic, rod-shaped elements; multitudes within a space of a hundredth of an inch. For many years their nature and function remained a mystery. Now the principal facts are established. These minute, rodlike bodies are bacteria; they may be isolated readily from the substance of the nodule and cultivated by themselves in the laboratory. Their name is as yet undecided. Some students, whose pleasure it is to decide whether appropriate appellations are imposed upon various objects, animate and inanimate, call them *Bacillus radicicola*. Others, with equal authority

and good intentions, designate them *Rhizobium leguminosarum*. As time goes on, it is not improbable that other names will be invented and inflicted upon these microscopic chemists, which seem to have solved Nature's great problem of restoring nitrogen fertility to the soil. Inasmuch as these lowly but extremely beneficent toilers of the living earth "fix" — that is, make — atmospheric nitrogen chemically available for plants, we may call them "nitrogen-fixing bacteria" in deference to their function in the economy of Nature. This name has no standing in scientific catalogues; hence it does not interfere with the orderly progress of bacterial nomenclature. It has the somewhat plebeian advantage of meaning something.

Quite unlike the history of the unsatisfactory attempts to name the nodule bacteria is the record of the experiments which led finally to the establishment of the nitrogen-fixing powers of the microbes.

It was found nearly three decades ago that the nodule bacteria could be cultivated in glass bottles containing sugars, mineral salts, and water, but no nitrogen-containing substances whatsoever. All forms of life previously studied required some form of combined nitrogen, either as nitrogen derivatives

CLOVER PLANT SHOWING NODULES UPON ROOTS

or nitrates for most plants, or as highly organized nitrogen, as proteins and their derivatives, for animals.

Here for the first time a living entity was revealed which could accomplish quietly, effectively, and without visible effort what man had tried to do with high temperatures, tremendous electrical currents, and powerful chemical reagents. It could be shown that the amount of nitrogen fixed by the activity of the microbes and recoverable from their growths in the glass bottles bore a direct relationship to the amount of sugar the microbes used for energy. Some cultures were found to be rather more active fixers of nitrogen than others. If the temperature became too low — much below summer heat — the process of fixation was slowed up or it even ceased. Too much heat, even a few degrees above that of the hottest days, killed the microbes very quickly. Precisely as the plants cease growing at the temperature of winter, so these microbes became quiescent, although fully able to grow when the summer sun again warms up the earth. Even more striking was the relation of growth of the nitrogen-fixing bacteria to the reaction of their surroundings. If the solution of sugar and salts was too acid — too sour, in other words

— the organisms immediately showed signs of inactivity. Farmers know that clover does not thrive upon a soil that has become "too sour." Too alkaline a medium also restrains the activity of the microbes, and every agriculturist knows that too much caustic alkali, as lime, used to correct sour soil, may do harm. In every way the nodule germs agree in the manifestations of their activity with the observations of the tiller of the soil.

The greatest discovery, after all, was the significance of the symbiotic relation between the clover plant, the nodules on its roots, and the microbes found within the nodules.

Careful studies of the quantitative fixation of nitrogen by clover plants, grown both in soil sterilized to kill all nodule bacteria, and in sterilized soil intentionally infected with laboratory growths of nodule bacteria, showed very clearly that the clover plant did not "fix" nitrogen appreciably in that soil which was freed carefully from all nodule bacteria. On the contrary, that sample of soil which was artificially infected with laboratory cultures of nodule microbes (first, of course, destroying all preëxisting microbic life to make the experiment conclusive) was found to be materially enriched in nitrogen after a crop of clover had been

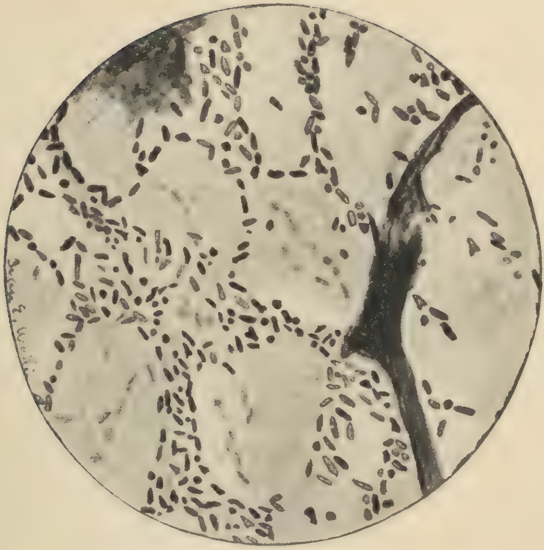
grown upon it and then ploughed into the ground. The nodules of clover plants grown in germ-free soil, furthermore, were poorly developed and contained no nodule microbes, whereas the nodules of clover grown upon soil purposely infected with the nitrogen-fixing microbes were luxuriantly developed and teeming with the organisms. It was also found that clover seed could be mixed with a culture of nodule bacteria just before planting and thereby mature a very satisfactory nodular growth.

The nodule bacteria are found in practically all soils where clover grows well, and usually it is unnecessary to add laboratory-grown cultures. There seems to be some evidence that suggests that different kinds of clover harbor slightly different varieties of nitrogen-fixing bacteria. Other leguminous plants have nodules of similar appearance and function, and, so far as available information goes, the same fixation of nitrogen by nodule bacteria may be ascribed to them.

The relation of the nitrogen-fixing bacteria found in the nodules of the roots of the *Leguminosæ* to the plant which shelters them is fairly well established. The plant furnishes the requisite sugars or starches for the energy requirement of the nodule microbe,

as well as the requisite moisture and mineral requirements, and a place for them to work. The microbe accepts the shelter and other perquisites, and, in return for all this, absorbs nitrogen from the air and unites with it hydrogen, carbon, and oxygen, forming nitrogenous substances which make the otherwise useless atmospheric nitrogen gas a very valuable constituent for the plant. When the fall comes, and the clover and other legumes cease to grow, the farmer "turns under" the crop, as he says. The nitrogen of the clover plant, including that fixed by the nodule bacteria, gradually becomes soluble in the upper layers of the soil, where it is available for those crops which do not fix the nitrogen they require for their structure and fruition.

Nowhere is the masterfulness of Nature more strikingly shown than in this remarkable copartnership of a lowly microbe and a highly organized plant. Neither acting alone can accomplish the desired end — that of fixing and making available the nitrogen of the air for plant life. Both acting together in good faith stand between the gradual loss of the supply of available nitrogen, which is swept into the sea, or removed from the farm as fruit, cereal, vegetable, or food for animals, and the



SECTION OF ROOT NODULE OF CLOVER PLANT SHOWING NUMEROUS NODULE (NITROGEN-FIXING) BACTERIA

perpetuation of vegetation upon the face of the earth. Fortunate, indeed, it is that Nature cares for her offspring, and shows those of mankind who are true students her marvelous secret processes for the perpetuation of life.

Gradually mankind is bringing aid to the nodule microbes whose activities are overtaxed in these days of intensive cultivation. At first Chile contributed, and still does contribute, that rich fertilizer known as *guano*, the remnants of exuberant bird life upon the dry and barren shores of northern Chile and neighboring islands. Also, man is harnessing the torrent and drawing therefrom the lightning, and utilizing this power to force chemical reactions to take place whereby nitrogen is united with such substances as carbon and hydrogen and oxygen to form nitrogenous food for plants. As the available cultivatable land decreases in proportion to the population dependent upon it, and therefore as the yield per acre must be the greatest possible, the artificial nitrogenous plant foods prepared by human agencies will largely supplant the microbic modicum. When this time has come and the nitrogen-fixing bacteria no longer stand between mankind and the exhaustion of the soil, the true student of Nature's marvels will still pay reverence to this

masterpiece of efficiency and constructive copartnership between plant and microbe.

Another element that is of some importance in the economy of living things is sulphur. Sulphur occurs in relatively small amounts in the tissues of plants, animals, man, and practically all known living things. When plants or animals or other animate beings die, the sulphur, as well as the nitrogen and carbon and other elements, is reduced to very simple combinations. In the former, hydrogen and sulphur form the foul-smelling gas, hydrogen sulphide. The peculiarly offensive aroma of an elderly egg is due in no small degree to this gas. Hydrogen sulphide also is found in some so-called "medicinal springs." In the minds of some, the thought of medicine immediately suggests something very disagreeable to taste and smell, just as the thought of germ or microbe or bacteria immediately suggests a hidden army of merciless death-dealing incitants of disease. Hence such springs are credited with mystic healing properties; for what ailments is not clearly understood.

The sulphur bacteria, very appropriately named, change this foul-smelling, fœtid gas, hydrogen sulphide, to sulphur, which is then further oxidized to sulphuric acid. This, of course, immediately

unites with some neutralizing substance, as lime, to form calcium (lime) sulphate. Sulphates are used by plants to form again the highly complex nitrogenous, sulphur-containing substance which is the living tissue. The transformation of the lifeless sulphate, together with nitrates, carbon dioxide (carbonic acid gas, as it is sometimes called), water, and other mineral substances, into plant tissue, under the action of the green coloring matter of the leaves (chlorophyll), marks the high point in the energy requisite for the cycle of life. The animals eat the plants; plants and animals die. The bacteria then change the useless, lifeless, but very complex combinations of nitrogen, sulphur, and other elements to the simple, mineralized compounds of these elements, and the flowing waters above and beneath the ground bring them back again to the roots of the plants, and thence to the silent, busy laboratory of the leaves where living nature reconstructs itself.

In a somewhat similar way bacteria change compounds of iron and carbon dioxide (ferrous carbonate) to the reddish-brown, insoluble mass which is known as "bog-iron" ore. Waters rich in this compound of iron are always detectable by the reddish sediments which the iron bacteria make.

Sometimes these iron microbes are troublesome to engineers; if water containing much iron in soluble form is passed through pipes, without some preliminary treatment to remove it, the iron bacteria grow therein and form deposits of iron oxide (iron rust) which may accumulate in tubercular masses of sufficient size to obstruct or even prevent entirely the flow of water.

Other essential but less conspicuous elements, as phosphorus, exhibit similar cycles between the living and the dead. The ancient custom of planting wheat or flowers upon the graves of the loved ones assumes beautiful significance in the contemplation of the cycle of life.

CHAPTER VII

Sewage and civilization — Bacterial purification of sewage — History of the development of sewage purification — Importance of bacteria in the process — Counting living bacteria — Purification of drinking-water by bacteria — Effects of biological filters upon the health of the community — How biological filters work.

THE farmer's wife knows that the drainings from the kitchen sink, in relatively large volume, may be poured upon the ground without creating a nuisance. The waste from man and animals may be scattered over the earth, and all traces of it will soon disappear. If the same wastes were allowed to decompose in a water-tight vessel, as a pail, they would soon become intolerable. What happens to these noisome substances when they are cast upon the ground? What part does the earth play in the wholesome disposition of these effete residues of life? The answers to these queries are of paramount importance, because the satisfactory disposal of domestic and manufacturing waste is a prerequisite of communal life.

The location of cities, towns, and villages upon watercourses in the pioneer days of our history was not a matter of chance. Transportation by water was an important consideration, of course; the

removal of human waste by water was also a determining factor. The sewage of the community was simply collected in sewerage systems and carried directly into the neighboring river or lake. The great dilution of this sewage, together with its natural purification by sedimentation and oxidization, reduced the menace of decomposing excrement to a minimum. The inevitable growth of cities sooner or later led to the discovery that watercourses could not be indefinitely laden with sewage. A time came when the oxidizing capacity of the waterway was exceeded, and unmistakable evidences of gross pollution indicated the limitations of water carriage as a means of sewage disposal. The smaller the watercourse in proportion to the population, the sooner this danger point was reached. Tidal waters usually cleansed themselves, however; the ocean is a vast reservoir into which an immense amount of filth may be emptied without noteworthy effect.

The unrestrained pollution of river water with sewage led to far greater danger than the mere creation of a nuisance. Cities and towns located upon lakes and streams were visited almost periodically by epidemics of typhoid fever. This was especially noticeable in Massachusetts, where densely

populated cities grew up on the banks of the Merrimac River. This stream rises in New Hampshire. New Hampshire towns, as well as Massachusetts cities, empty their sewage into this beautiful waterway, which was a clear, pure, and beautiful river before the habitations and industrial establishments of man defaced its banks. The Merrimac River not only receives the sewage of several large cities; it furnishes their drinking-water as well. The cycle of contamination and infection, therefore, is quite simple and direct. Sewage is emptied into the river and drinking-water is drawn from the river. The river water dilutes the sewage of the upstream towns, and the sewage of the upstream towns pollutes the drinking-water of the communities farther down. Thus, typhoid bacilli from the sewers of Lowell travel with the current to the intake of the Lawrence water-supply. The health authorities of Lawrence, situated twelve miles below on the Merrimac River, could predict almost to a day when typhoid would appear in epidemic proportions, following an outbreak of the disease in Lowell. This situation was not uncommon throughout the eastern United States.

It became quite evident that some satisfactory and safe method of disposing of sewage must be

developed; otherwise the future of many towns and cities bordering upon rivers and lakes was jeopardized. The first steps toward a solution of this extremely vital problem of sewage disposal were taken in England, where the same condition, but in even more acute form, had existed for many years.

In 1868, the Rivers Pollution Commission of the London Metropolitan District carried out some experiments upon sewage purification, with results which were not well understood at that time. Several tubes, eight or ten feet in length, open at each end, were placed in an upright position and filled with various insoluble substances, each separately. Garden earth, fine and coarse sand, gravel, small stones, and coke were used. Small amounts of sewage were poured upon the tops of these "filters," and allowed to trickle through the entire length of the column of material and out at the bottom. Chemical analyses of the inflowing sewage and the outflowing liquid revealed the unexpected fact that a not inconsiderable change had taken place in the soluble organic constituents of the sewage during its relatively brief sojourn through the filter. The changes mentioned indicated that a distinct simplification of the complex, noxious constituents of the sewage into relatively innocuous,

harmless mineralized combustions of the elements involved had occurred. Not very much was accomplished beyond this point, however, and the solution of the problem of sewage purification was reserved for another time and place.

The interval between these important but incomplete experiments of the Rivers Pollution Commission with their tubes filled with soil, stones, and sand, and the publication of the epoch-making studies upon the purification of sewage performed in the Lawrence Experiment Station located upon the Merrimac River, was an important one in the history of civilization. There were no great wars, to be sure; no mass movements are recorded by historians as milestones in the story of mankind. No national or international heroes received publicity or attained notoriety; no particularly significant political issues stirred the complacency of the proletariat. Nevertheless, communal existence was on trial; the future of the great and ever-growing city was being weighed in the balance of fate. Mankind was in danger of being decimated, or at least of being dispersed, by the accumulation of his own domestic waste.

The problems of sewage disposal and of the procurement of pure water in adequate volume for

domestic or factory needs were horns of a dilemma in city administration which required a satisfactory solution, if large numbers of people were to live together in comfort and safety. Once again Science turned to Nature for inspiration and guidance. That same "living earth," which quietly decomposes the kitchen waste of the farmer's solitary house, was set at work upon the massed waste of the city. The simplicity and directness of the process is characteristic of naturally occurring phenomena.

The scientists at the Lawrence Experiment Station, mindful of the experiments of the Rivers Pollution Commission, erected a series of watertight tubs or vats, each about eight or ten feet in diameter and six feet deep. These were filled from the bottom upwards, first with a six-inch layer of stones, the size of a hen's egg; then an equal layer of coarse gravel. This was to permit of free drainage. Upon this drainage layer was placed about four feet of carefully screened sand. In some tubs the sand was replaced by garden soil, but it was found that the passage of fluid through this was too sluggish to accomplish the desired end. Consequently, this was discontinued.

Sewage in definite volume was poured evenly

upon each filter; its downward rate of passage was regulated by the manipulation of a valve placed at the outlet. The sewage was, therefore, forced to seep slowly to the outflow pipe. Many experiments were necessary to determine the proper rate of flow. Chemical analyses of the sewage applied at the top of the filter, and of the liquid drawn off at the bottom, showed the nature and extent of the changes which took place during its sojourn there.

A number of essential details in manipulation were discovered which, when carefully observed, led to important results. At the start, it was found advantageous to fill the filter carefully with sewage, the outlet being closed, and then to allow the sewage to remain in undisturbed contact with the sand for some hours. This was much more essential to success when the filter was used the first time than subsequently. Also, after the filter was "conditioned" in this manner, it was found necessary to allow a period of rest to intervene between fillings. If this latent interval were not observed, the filter eventually became foul and offensive, practically as the ground around the waste-pipe from the kitchen sink becomes foul and offensive if the housewife throws too much sink washings upon it without giving it a rest. In either case, the sand or

soil becomes waterlogged and sewage-clogged, and thereby loses the ability to effect purification. The amount of sewage that could be taken care of in a day's time was found to be definitely limited. Too much sewage on the filter was found to be quite like too much food for man — a forerunner of indigestion.

When, however, the filter was running properly, with proper preparation, proper dosage, and sufficient rest between applications, the results of its action were almost unbelievable. Consider the contrast! Foul-smelling, turbid, disgusting waste of human activity, known as sewage, applied to the top of a four-foot column of sand in a wooden tub: clear, limpid, sparkling spring water flowing in a thin stream from the bottom of this four-foot layer of sand.

One of the regular demonstrations which has been contrived for visitors to this abode of miracles is to place side by side in drinking-glasses some sewage taken directly from the top of the filter and some of the sparkling effluent flowing from the pipe which drains the filter. Both are drawn in the presence of the guest. Of course, one wholly unfamiliar with Nature's method of purification would see at once the striking difference between

the sewage and the effluent of the sewage filter. It requires, however, an unusual degree of courage in the casual visitor to induce him to drink of the sparkling water whose origin, scarcely four feet above, is so foul and disgusting. Those resident in the Experiment Station, however, never failed to drink deeply and with genuine relish of this purified sewage.

Not only by sight, smell, and taste was this remarkable transformation demonstrable, but the success of the process was equally convincing from the nature of the chemical changes which occurred. The organic compounds which confer upon sewage its obnoxious characteristics were found to be changed quantitatively into fully mineralized and wholly unobnoxious substances, similar to those found in spring water; none of the elements present in the original sewage were missing in the effluent, however. Even after several years' operation, none of the nitrogen impurities remained in the filter. That is to say, there was no storage of the ingredients of sewage. It was finally discovered that the entire process is one of bacterial digestion.

Precisely as man and animals eat meat and other protein, digest it in the stomach and intestines, and provide thereby the necessary food to carry on

their daily work, so the microbes resident in the filter take into their bodies the organized nitrogen-containing substances of the sewage, and derive from them the requisite food to carry on their daily work.

The residue which man eliminates after using the digested food for his sustenance is an important constituent of sewage. It is no longer of use to man, and it is necessary to get rid of it so man may live properly. Also, the waste of man, containing very valuable substances for the plant, is not in a suitable form for plants to utilize, and large losses would occur if Nature did not step in to prevent this waste from being lost to the plant kingdom. Fortunately, among the soil microbes there are those which find in domestic waste precisely the food they need, and therefore they take up the white man's burden, as it were, and extract therefrom their own food. Like man, the soil bacteria leave a residue containing nitrogen, representing their own waste. This nitrogen, however, is linked to oxygen, as nitrates, and in this form it is fully mineralized and wholly unable to create a nuisance. Furthermore, it is entirely suited for the use of plants, which can unite this nitrogen, through the energy of the sun's rays, with water and carbon to

form new plant tissue, the support of animal and human life.

This purification of domestic waste, therefore, is not only a prerequisite for communal existence, but it is also a factor of prime importance in the cycle of living things. The time must come, however, when microbic digestion will be too expensive for the purification of the sewage of cities containing millions of inhabitants. Even the most efficient of modern sewage-disposal plants require much space for their construction. Land around large cities is too valuable to be devoted to this purpose. Some new plan must be worked out for the disposal of the ever-growing menace of domestic waste. Presumably in the future, therefore, bacteria and the living earth will play a much less conspicuous part in the disposal of sewage, even as they are being supplanted to-day in the fixation of nitrogen. This will not detract from their unseen and all but unrecognized participation in the history of human communal existence.

The earliest studies upon the purification of sewage by bacterial filters were restricted to a chemical comparison of the inflowing sewage with the outflowing, purified effluent of the filters. With the rise and development of the science of Bacte-

riology, however, the importance of the microbes became recognized, and no inconsiderable part of the fame of the experiments at the Lawrence Experiment Station rests on the discovery of the effects of sand filtration upon the numbers and kinds of bacteria in sewage and water.

One of the most significant changes in sewage during its passage through the sand filter is the great reduction in the number of microbes in the outflowing water in comparison with the number of microbes in the unpurified sewage, some four or five feet above, on the top of the filter. Also, the kinds of bacteria found in the effluent of the filter are quite unlike those of the raw sewage, both in number and in the proportions of the various kinds. Fully ninety per cent or more of the sewage microbes are left behind during the filtering process.

Inasmuch as bacteria are very small, indeed, in comparison with the spaces between and around the sand grains of the filter — spaces which afford a ready passage to particles as minute as those of finely divided clay — it is obvious they are not merely strained out and withheld by the filter. As a matter of fact, the majority of sewage bacteria, pathogenic and non-pathogenic, are destroyed,

even as the soluble organic constituents of the sewage are destroyed.

The final demonstration of the effects of filtration upon sewage bacteria awaited the development of new methods in Bacteriology, methods which permit of the accurate enumeration of bacteria in the sewage itself. At first sight, it would seem beyond the range of possibility to determine the number of bacteria in a sample of sewage. It is possible, however, to determine the number of bacteria not only in sewage, but in any liquid, by means of a very simple and fairly accurate process of serial dilution, even when millions or hundreds of millions of microbes are present. A concrete example will indicate the process

The unit of liquid measurement used in scientific procedures is the cubic centimetre, about one-thirtieth of a fluid ounce, or about twenty average-sized drops. To count the number of bacteria in a fluid containing large numbers of bacteria, as a sample of sewage (which commonly contains from five to ten millions of bacteria to each cubic centimetre), a cubic centimetre of it is removed with a sterile measuring tube and is added to ninety-nine cubic centimetres of sterile, germ-free water. This is shaken. The microbes are thereby

uniformly distributed in one hundred times the volume of fluid they originally occupied. Each cubic centimetre consequently contains one one-hundredth of the original number. One cubic centimetre of this one-to-one-hundred dilution of the original number of bacteria is added to another ninety-nine cubic centimetres of sterile water. Each cubic centimetre now contains one one-hundredth of the number just added, or one ten-thousandth (100×100) of the number in the original sample. If the process is repeated once again, each cubic centimetre of the last dilution will contain one millionth ($100 \times 100 \times 100$) of the original number of bacteria in the original cubic centimetre of sewage.

The next step is to count the microbes in the equivalent of one millionth of the cubic centimetre started with. The method usually followed is to add some of the final dilution to a culture medium in which the microbes can grow. Gelatin is such a medium. It has the peculiar and important physical property of becoming fluid when it is heated to about 90° F., and of again becoming firm when it is cooled to about 70° F. Gelatin is also a good food for bacteria.

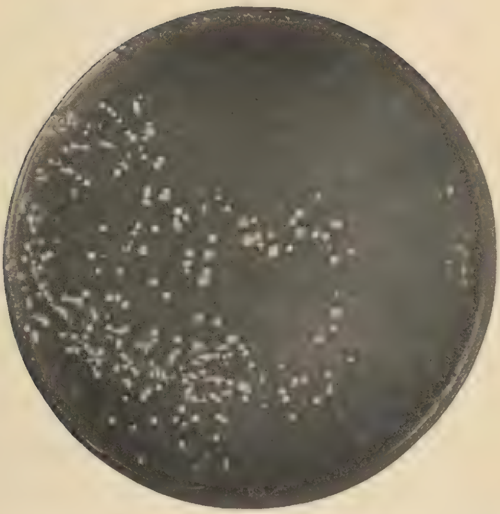
The procedure for counting is very simple in the

light of these few details of accurate dilution and of a nutrient culture medium that becomes fluid when warm and solid when cooled. A small amount of germ-free gelatin, about ten cubic centimetres, is warmed to the point of becoming liquid. A cubic centimetre of the diluted sewage, made as just described, is added to it, being careful not to allow outside microbes to get in. The gelatin and added bacteria are then thoroughly mixed to insure a uniform distribution of the microbes, and the entire volume is then poured into a shallow, sterile glass dish, having a cover which prevents access of dust or other impurities. The gelatin is cooled rapidly to the point of solidification, and the bacteria are then immobilized in place, precisely as stones are frozen in a puddle of water. They cannot move about, but they can and do grow. Their descendants accumulate immediately about them. Millions of descendants thus kept in one place become very clearly visible to the naked eye, where the original individual microbes could not be seen except with the very highest magnification of the microscope. Even within one day, they are clearly visible as spots scattered throughout the gelatin. Each spot of growth, or colony as it is appropriately called, represents one

microbe of the first generation. A count of the colonies on the plate multiplied by the dilution will give a very good estimate of the number of living bacteria present in the original sample. In the instance cited, if seven colonies grew in the one-to-one-million dilution, the initial number of bacteria was obviously seven million per cubic centimetre.

By this method of counting bacteria and by procedures equally direct, the fact was established that the purification of sewage by bacterial digestion altered the chemical constituents and also reduced the number and kinds of microbes in it. The last-named factor is vitally important to mankind; if strong sewage can be changed both chemically and microbially by filtration into a relatively innocuous fluid, why not attempt to purify dilute sewage, such as the waters of many American lakes and streams, by filtration, and thereby effect the removal of microbes which are, or may be, a menace to health? Sand filtration was tried and the results were found to be very satisfactory.

Among the earlier sand filters erected for the purification of water destined for drinking and general domestic use were those built at Lawrence, Massachusetts. It will be recalled that Lawrence is located on the banks of the Merrimac River. This



CULTURE PLATE SHOWING BACTERIA DEVELOPING FROM ONE TEN-THOUSANDTH OF A CUBIC CENTIMETER OF SEWAGE

Each white spot is a colony, the twenty-four-hour progeny of a single microbe. The culture medium is gelatin

rimac River water. The tap in question was discontinued, and only filtered water was made available for drinking. The epidemic ceased, and no further typhoid cases appeared among the hundreds of workers in that particular mill.

The true secret of the mechanism of the purification of sewage by sand filtration was not revealed until a careful microbic study was made of the sand filters themselves. It was discovered that the layer of sand is practically inert. All of the vital processes take place in a thin, delicate film of microbes which forms a continuous layer — a living carpet, as it were — upon the top of the sand. This layer is called a *Schmutzdecke* by the Germans, and no English word has been coined to replace this appellation. When the *Schmutzdecke* is undisturbed and continuous, the filtering process is satisfactory. The sewage or impure water and the contained pathogenic bacteria must pass through this broad microbic stomach; the soluble chemical constituents of the sewage nourish the filter microbic population and are thereby reduced to simple, mineralized substances available for plant food. The pathogenic bacteria cannot pass through the film, and they perish.

An unfortunate accident revealed the disastrous

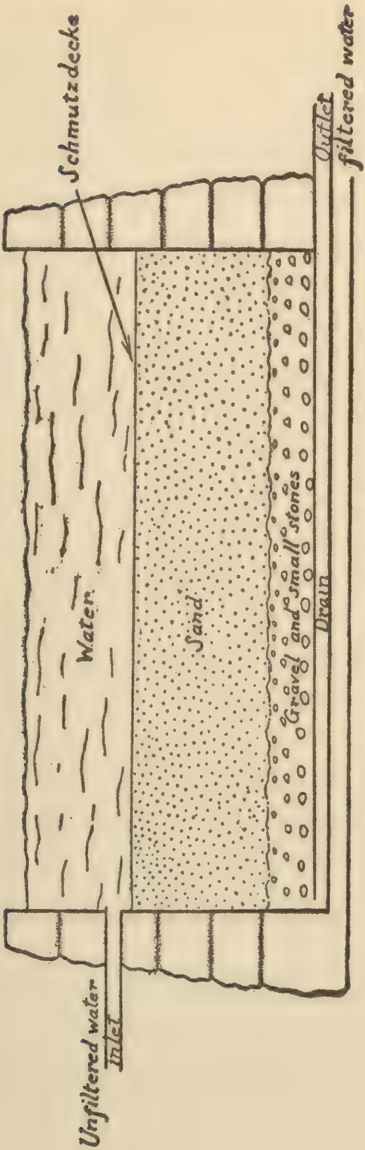
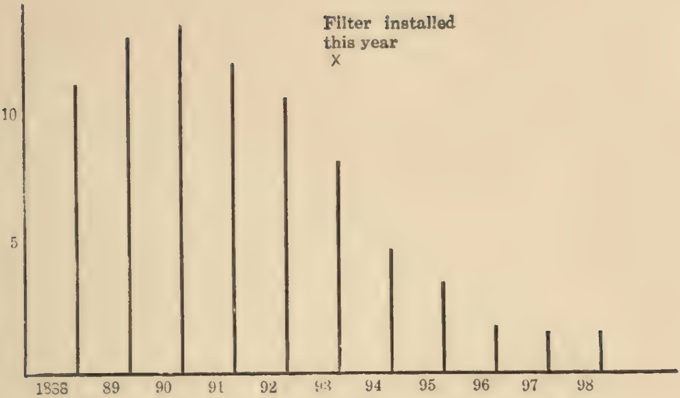


DIAGRAM OF WATER FILTER

consequences of breaks in the continuity of the *Schmutzdecke*. The unfiltered river water is maintained at a constant level of about three feet above the Lawrence filter by automatic float valves. One cold day in mid-winter, when an epidemic of typhoid was raging in Lowell, the water level on top of the filter was reduced by some accident not clearly understood to only two inches. It remained at this low level for several hours. Ice formed and froze the water solidly to the top layer of the sand. When the normal depth of three feet was restored, the ice floated up, but carried the topmost strata of the sand in the filter, including, of course, the *Schmutzdecke*, with it. The continuity of the microbic carpet was broken, the impure and undigested water flowed into the city mains, and, after the predictable latent period, a severe epidemic of typhoid appeared, well scattered throughout the city. Suitable mechanical devices were installed to prevent a recurrence of this unfortunate interference with microbic continuity on the filters, and typhoid fever in epidemic form has never reappeared.

The bacterial purification of water by sand filtration is destined to become obsolete. Only two or two and one half millions of gallons can be purified



Cases of typhoid fever per 10,000 inhabitants, Lawrence, Mass.

Cases of typhoid fever per 10,000 inhabitants, Zurich, Switzerland

successfully upon each acre of filtering surface, and much less if the water is turbid. Additional filter space must be provided for periods when the filter has to be out of operation, and land is too valuable around large cities for this purpose.

Chlorine gas is extensively used to eliminate pathogenic bacteria from reasonably clear waters. Much less than one part of chlorine to a million parts of water will effectively destroy pathogenic bacteria. This problem of safe, potable water is, therefore, more readily solved than that of the disposal of communal waste. While mankind is, therefore, supplanting microbic purification of water by chemical means, the part played by the *Schmutzdecke* in safeguarding the health of large communities in their younger days cannot be overlooked in the history of civilization.

CHAPTER VIII

Bacteria and the industries — The retting of flax — The tanning of leather — The manufacture of synthetic rubber — The manufacture of vinegar — Bacterial diseases of plants.

One of the least conspicuous, but certainly one of the most important, functions of the bacteria in nature is the part they play in the regulation of the cycle of life upon the earth. They tear down those highly organized structures from which the spark of life has departed; they salvage the valuable building materials contained in these structures, and reissue them to the plant kingdom in proper condition for reconstruction into new living substance. Bacteria also transform the waste products incidental to life-processes into utilizable substances, and replace, through the activity of the nitrogen-fixing organisms, some of the unavoidable nitrogen losses.

There are many other fruits of microbic activity which are of importance to man. Some of these are in association with the preparation of food; some are related to the development of particular flavors or desirable changes in foods; many are exclusively commercial, technical, or industrial processes in which microbes are utilized as living chemical

reagents to induce changes that man has failed to accomplish by any other means. Among these procedures are some of great antiquity; others have been developed under the stress of practical need during the World War.

THE RETTING OF FLAX. Among the most venerable of microbic services to mankind is the retting of flax. The capable housewife, at the time when Egypt was at the height of its glory, took pride, even as to-day, in the possession of fine linen. No other fabric has ever filled exactly the place linen occupies, even though it may have been more expensive.

The threads from which the linen is woven are spun from the prepared fibers which occur in long strands in the flax plant. The first step in the manufacture of threads of flax, therefore, consists in liberating these long fibers from the stalks of the mature plant itself. The process is known as "retting," which means literally rotting. It is quite simple, and very little improvement has been made in it since primitive man discovered that ripe flax stalks, carefully placed in parallel bundles to prevent injury to the fibers, could be immersed in running streams and ponds for a period of time to

loosen the substance which surrounds the fibers, and cements them together. Usually several weeks' immersion are required to attain the desired degree of loosening of these strands of fibrous material. At the proper time, the bundles are removed from the stream and spread in thin layers to dry in the sun. The woody and gummy substances of the stalk break off, and the residual fragments are detached readily by passing the partially freed fibers between rows of pointed nails or wooden combs. If the process has been carefully carried out, the fibers are silky, slightly brownish in color, strong, and very pliable. They may be spun into threads of great strength and of exquisite fineness.

In some countries, where running streams or ponds are unavailable, the bundles of flax are spread thinly upon the ground. The sun, the rain, and the dew effect the same disintegration of the woody substance of the stalks as the water immersion, and leave the fibers in a readily separable state. It is essential that the bundles of flax be turned over every few days to insure uniform results. This method of "dew retting," as it is called, requires much closer observation and more skill to carry out successfully than the water retting. If the process is arrested too soon, the separation of

the fibers from the stalk is only partly accomplished. If the exposure is too long, the strength of the fibers is definitely lessened.

Several other fibers of importance — jute, hemp, and rami — require a preliminary treatment akin to retting to loosen the woody substance of the stem or leaf, and thus liberate the long bast fibers which are so useful in the manufacture of thread, cord, and even rope.

The process of retting is ordinarily ascribed to bacterial action. The flax fibers are resistant to microbic digestion, but the gummy substance of the flax stalk is quite readily digested. It is stated that in Belgium and Holland, where much of the finest retting is done, certain streams are better adapted to the retting process than others. This is attributed to the fact that the microbic population of these watercourses varies somewhat. Peculiarly adaptable kinds may preponderate in some streams, and be absent, or only present in small numbers, in neighboring streams. This, however, is not by any means an established fact.

Efforts have been made to increase the effectiveness of the bacteria which play a part in retting. Especially designed tanks have been made which not only will mechanically aid the process through

the removal of harmful products which result from the action of the microbes, but will also secure more uniform exposure of the individual flax stalks to the microbes themselves. Thus far, however, notwithstanding several patents, Nature's method has much the advantage.

Attempts have been made also to liberate the flax fibers by chemical action, but as yet none are successful. It has been found that any chemical powerful enough to loosen the substance of the stalk will at the same time weaken the flax fibers and destroy their luster.

THE TANNING OF LEATHER. Another industry of great importance, whose origin is lost in the fragmentary record of primitive man, is the preparation of hides of the larger herbivorous animals for the use of mankind. From time to time improvements have been introduced in the various processes necessary to reduce the freshly removed hide to the fixed and durable condition known today as "leather," but the essential features of the process are those practiced by the artisans of the earlier civilizations. The leather industry is dependent upon the activities of several kinds of microbes for its success.

The skin of most animals consists of three layers. The innermost of these, when properly treated and hardened, is called "leather." The first step in the transformation of the hide into leather is the removal of hair and the outer layers of the skin. The dried hide is first of all soaked thoroughly and kept in a moist place to allow the tissues to soften. The fragments of flesh adhering to the hide, together with the outer skin (which swells and partly dissolves in the water), furnish food for microbes which are always present. These digest the undesirable portions of the hide to such an extent that their removal is readily accomplished. The inner layer of the skin is left unchanged, unless the microbic digestion is allowed to continue for too long a time.

In recent years it has been found advantageous to substitute a dilute solution of lime for the bacterial digestion of the outer skin layers. The hides are immersed in the lime bath for some time. The undesirable substance is thereby so altered that it may be removed readily by scraping. After the scraping is done, and the innermost layer of the hide is freed from all foreign material, it still contains lime. This must be got rid of; otherwise, the finished product — the leather — would be stiff

and brittle. The lime is readily dissolved by weak acid solutions. Bacteria produce acids from starchy substances, such as bran or starch. Therefore, a bran bath is made, into which the limed hides are placed. The microbially produced acids unite with the lime, forming lime-acid compounds (calcium lactate, principally) which are soluble in the water. In this simple manner the lime is removed.

The question naturally presents itself, Why not add the acid — the lactic acid — directly to the limed hides, and thus speed up the process rather than wait for the microbes to generate the acid after the hides are placed in the bath? In the first place, pure lactic acid is too expensive to use; it is difficult to separate it from other substances which are produced along with it. Sea water contains gold, but it costs much more to separate the gold from it than the small return of the precious metal would justify. Similarly, lactic acid occurs in relatively small amounts in the fermenting bran; the process of generation by microbic action is not costly, and it works very well, indeed. Experience has shown, too, that the gentle solvent action of the slowly forming lactic acid in the bran bath is more effective in dissolving lime than corresponding amounts of pure lactic acid would be.

The subsequent treatment of the cleaned hide varies with the purpose for which it is destined. The soft, pliable leather known as "chamois" is treated with oil. Other kinds are subjected to a variety of processes, which are usually chemical rather than microbic.

Hard, tough leather intended for the soles of shoes is prepared by a process known as "tanning." The prepared hides are hung side by side or laid flat in deep pits. Between each two hides is a thin layer of hemlock bark cut into small pieces, together with finely crushed nutgalls, and other substances. The pit is then filled with water. After several days, or even weeks, the hides are withdrawn and placed in another pit prepared in the same manner. During the process the liquor in the pit becomes quite acid and dark-colored. The "tannin," drawn from the hemlock bark, gradually sours, and there is considerable evidence that this souring is due to microbic activity. The souring seems to facilitate the solution of the tannin from the hemlock bark (tanbark) and it also assists in the combination between the tannic acid and the gelatin-like substance of the hide. It is this tannic acid, gelatin-like combination which makes the hide tough, rather flexible, and very durable.

Various expedients have been tried to hasten the entire process of preparing and tanning hides, but none so far devised can compete in excellence with the good old-fashioned process where the tanner and his hidden and unrecognized microbic associates worked together to produce real leather, enriched by a pride in its excellence and durability.

Artificial Rubber. An important and comparatively new discovery is the manufacture of artificial or synthetic rubber. Rubber was used in vast quantities during the World War, and there were indications that the supply of natural rubber might be insufficient for the needs of the world. Much time and money were spent in attempts to prepare a suitable substitute, upon a commercially feasible scale.

After considerable experimenting, a very cheap and readily obtainable substance, starch, was chosen as the starting-point for the new process. The starch was first fermented by peculiar microbes which form considerable amounts of a product known as "butyl alcohol." No purely chemical method is known whereby butyl alcohol can be made cheaply. It contains carbon (C), hydrogen

(H), and oxygen (O) in the proportions indicated in the following chemical formula:

$$CH_3.CH_2.CH_2.CH_2OH, \text{ or, } C_4H_9OH$$
Butyl alcohol

The butyl alcohol, obtained from the fermentation of the starch (almost any kind of starch will answer), is treated with other chemicals which have no place in this discussion, because they are not of bacterial origin, and the butyl alcohol is thereby transformed into a substance known as "isoprene." The chemical formula for isoprene is somewhat more complex than that of butyl alcohol, as is indicated below:

$$H_2C : C.CH_3.CH : CH_2$$

By comparatively simple subsequent procedures, this isoprene is converted into a very good grade of rubber which is chemically and in other respects similar to natural rubber. It has been stated that this artificially prepared rubber costs less than fifteen cents a pound to manufacture when large amounts — tons — are made at one time. This claim is apparently too hopeful. It is not feasible at present to produce it on a commercially successful basis. Artificial rubber does, however, represent a scientific achievement of great significance.

THE MANUFACTURE OF VINEGAR. The manufacture of vinegar is an important industry in which bacteria play an indispensable part. Vinegar, chemically considered, contains as an essential ingredient a considerable amount of a substance called "acetic," or vinegar, acid. This acid contains three elements, carbon, hydrogen, and oxygen, in the amounts and in the order indicated in the formula $CH_3.COOH$, where each of these three elements is represented by its initial letter. The mother substance from which acetic acid is formed is ethyl alcohol, which has the following chemical formula: $CH_3.CH_2OH$. Its formula usually is written C_2H_5OH, but the first form is preferable because the nature of the change from alcohol to acetic acid is more readily comprehended. It is as follows:

$$\underset{\text{ethyl alcohol}}{CH_3.CH_2OH} + \underset{\text{oxygen}}{O_2} = \underset{\text{acetic acid}}{CH_3.COOH} + \underset{\text{water}}{H_2O}$$

It is obvious from the chemical equation that an abundant supply of oxygen is a matter of necessity. The bacteria which transform ethyl alcohol to acetic acid are very appropriately called "acetic acid bacteria." They add this oxygen to the alcohol, or, as the chemist states it, "oxidize" the alcohol to acetic acid.

The three factors required to make vinegar acid, therefore, are clearly alcohol, acetic acid bacteria, and oxygen. The latter exists in the air to the extent of nearly twenty per cent; therefore, a supply of air is used in preference to pure oxygen gas, which would be extremely expensive. Pure oxygen gas would also kill the bacteria.

In practice, a heap of shavings, which offers a large exposure of surface to the air, is infected with acetic acid bacteria. These are obtained from "mother of vinegar," known to every good housewife living on a farm. A dilute solution of alcohol is sprayed upon the top of the heap of shavings (usually beechwood shavings are used because they do not add color or taste to the vinegar) by an automatic device. This trickles slowly downward in a continuous, thin film. It meets the acetic acid bacteria and the oxygen of the air, and it is thereby changed to vinegar. A tub at the bottom of the shavings catches the vinegar. The process is practically automatic, once the correct rate of flow of the alcohol over the shavings is obtained.

The alcohol may be obtained from cider, fruit juices, honey, molasses, or other natural substance containing sugar. Yeast, which may occur spontaneously in the fermentable juice or be added

intentionally, changes the sugar to alcohol. In this form the dilute alcohol solution is ready for oxidization to vinegar by the acetic acid bacteria.

Extraneous microbes, from the air or dust, may interfere with the action of the vinegar bacteria; hence it is customary in large factories to enclose with a wooden wall the heap of shavings upon which the acetic acid bacteria are growing. Air must be admitted freely, of course, and in the system just mentioned the air is forced upward from the bottom by suitable fans or pumps. The ascending air is thus brought into intimate contact with the descending film of alcohol.

The housewife frequently adds "mother of vinegar" — that scum that forms during the vinegar-making process in the home — to cider or honey or fruit juice, and thereby establishes her own vinegar factory. The scum contains acetic acid bacteria. It forms at the surface of the barrel or tub because it is at this place that the requisite air is available.

If flies, moulds, and dust are excluded, the home-made vinegar is as good as can be purchased. The only drawback is the slowness of the process. If some home-made device for furnishing air in sufficient amount to hasten the process could be found,

it would be a replica of the factory method of manufacture.

THE BACTERIAL DISEASES OF PLANTS. Brief mention should be made of the microbic diseases of plants. Apple, pear, and other orchard fruit trees are sometimes severely damaged by blight. This is a disease characterized by a rather abrupt blackening of leaves, twigs, and eventually the larger branches and trunk. The general appearance of an infected tree suggests at first sight the effect of fire. Indeed, the popular name for this disease is "fire blight." A closely related group of microbes has been discovered which causes these blights of the different trees. The bacteria are carried apparently from tree to tree by winged insects, particularly beetles and bees. Naturally, the first sign of blight in an orchard calls for prompt action. Tools used for cutting away diseased limbs must be carefully freed from the blight microbes before they are used on sound, healthy trees.

Another important group of plant diseases is that of the wilts. The vines of the cucumber, squash, pumpkin, the cantaloupe family, and the potato and tomato plants, may each turn yellow, droop and die. The microbes that cause this

rapidly spreading infection form a closely related group, both chemically and in appearance. It is supposed that the microbes are carried upon the legs and bodies of the insects, cucumber beetles, potato beetles, and others, which infest the various plants. The bacteria are rubbed off from the insect as it walks over the plant, and then gain entrance to the tissues of the plant through minute abrasions in the stem, or by direct injection brought about involuntarily by the insect enemy of the plant. Diseased plants should be burned at once, and an insecticide used to eliminate the insects.

The soft rot of the cabbage, turnip, carrot, and various kinds of melon, unlike the blight and the wilts, is usually an infection of the edible portion of the plant instead of the foliage. Here again microbic enemies are the direct cause of the trouble. Early and complete destruction of infected plants is the only method of correcting the spread of the disease.

Flowering plants as well as those cultivated for food are frequently the victims of different kinds of bacterial infection. Some of these spread rapidly and cause much loss. Several years ago an attempt was made to use a microbic enemy for the exter-

mination of the water hyacinth. This plant grows rapidly in shallow streams in certain parts of the country where, in spite of its beauty, it becomes a nuisance. Indeed, in several instances streams have been so filled with hyacinth growth that they have become mere swamps. A bacterial enemy of the hyacinth, which at times has caused great destruction of cultivated varieties, was tried out as a possible exterminator of the uncultivated hyacinths. The attempt was wholly unsuccessful. Other factors than plant and microbe must be involved to permit of a widespread destruction of the plant by its microscopic foe. In other words, a state of balance exists between the destructive activity of the microbe and the resistance of the plant to its attack. Under natural conditions (which man frequently disturbs by methods of artificial cultivation, and the like) the plant is stronger and survives. Until these balances between plant and microbe are fully understood in all their complexity, the mere sprinkling of myriads of microbes upon plants to be eliminated will be of little or no avail. In nature, the balance of survival practically never swings far enough in favor of the microbe to cause the extermination of the plant. Otherwise the plants would have perished long ago.

Also — and this is important to remember — had any kind of microbe succeeded in exterminating a plant, the microbe would in all probability have perished ignominiously with its plant host.

CHAPTER IX

The disintegration of animal and vegetable remains by bacteria — Lactic acid Nature's preservative — Its chemistry — The history of the preservation of milk by lactic acid — Lactic-acid bacteria of the alimentary canal of man — Lactic-acid bacteria and the churning of cream — The part played by lactic-acid bacteria in the preparation of sauerkraut — The preservation of ensilage by lactic-acid fermentation.

THOSE fortunate virile men who spend a portion of each year in the forests will remember where the moss-covered sylvan giant lies. Summer after summer it becomes somewhat more deeply covered with vegetable mould, and each succeeding year its snow-covered mound is somewhat less distinguishable. All vestiges of a dead animal, with the exception of the skeleton, disappear within a few weeks. Furthermore, the earlier stages in the disappearance of an animal are frequently detectable through the sense of smell even before they are discernible to the eye. The slow decay of the tree does not give rise to offensive odors.

The chemistry of the dissolution of animal remains is strikingly similar to the chemistry of the digestion of meat in the alimentary canal of man. Both take place in stages. The initial cleavage of

the complex substance of meat takes place in the stomach. This stage of the process is commonly referred to as gastric digestion. It is a combination of mechanical grinding and chemical disorganization. It reduces the meat to a semi-fluid, partially disintegrated mass which is passed on to the intestines where the final reduction into simple fragments is completed.

The process may be likened to the destruction of a brick building. The first step is to break the walls into pieces that can be removed from their resting-place. This destroys the architecture of the building. The final step is the separation of the individual bricks, which are then available for reconstruction into other brick edifices. The meats consumed by man for food do not correspond in their architecture to the human structure; the biological elements from which meats are formed, known as "amino acids," are bricks that may be built into the human frame. Digestion in the alimentary canal frees the bricks from their unhuman architectural design, and makes them available for reconstruction into the image of man.

In quite a similar manner bacteria reduce the flesh of animals into the building stones of which it is composed, and thereby replenish the stock of

building materials for Nature's great architects, the green plants.

The flesh of animals is rich in nitrogen-containing substances; far richer than plants. The total amount of nitrogen that could be recovered from an ox, for example, would be considerably greater than the total nitrogen content of an unusually successful acre crop of wheat. Inasmuch as the supply of nitrogen available for the cycle of life is much less than the corresponding supplies of carbon, hydrogen, and oxygen, it is quite apparent that the rapid decomposition of animal tissue is more significant than the slow decay of plant tissues in the economy of Nature.

Plant substance, however, contains disproportionately larger amounts of non-nitrogenous substances, as cellulose (woody fiber), starches, and sugars than of animal tissues.

It will be recalled that the bacterial decomposition of nitrogenous substances, as the proteins of meat, gives rise to elementary compounds rich in nitrogen. Ammonia is an important product of this bacterial action. Ammonia is an alkaline substance; therefore, the final reaction of the bacterially decomposed animal remains is alkaline. The bacterial decomposition of starches, sugars, and cel-

lulose, on the contrary, gives rise to acid products which contain no nitrogen. Acids retard the action of bacteria upon the nitrogen-containing substances which make up the bulk of the tissues of animals, and which are also important, although materially less conspicuous constituents of plants.

Plant decay, therefore, is a microbic process characterized by the formation of considerable amounts of organic acids. Among these acids is lactic acid, one of Nature's most important preserving agents.

Lactic acid, chemically considered, is somewhat more complex in its structure than acetic acid (vinegar). Its relation to acetic acid is indicated in the diagram below, using as before the initial letters of the elements, carbon, hydrogen, and oxygen, to describe their relations in space to each other and the subscript figures to indicate the amounts of each, above one, wherever the element occurs in multiple.

$CH_3.COOH$	$CH_3.CHOH.COOH$
Acetic acid	Lactic acid

Lactic acid, biologically considered, is a very widely distributed substance. It is formed very commonly wherever microbes and sugar solutions, or, in general, when microbes and carbohydrate solutions are brought together in nature. Lactic acid is the substance that gives the property of

"sourness" to milk. The great group of microbes that form lactic acid from sugars is widely distributed. Many of these are important agents in the preservation of human foods.

The housewife knows that "soured milk" keeps well. That is to say, it remains wholesome for a considerable time, and does not readily undergo foul-smelling, offensive putrefaction. Soured milk, in other words, contains its own preservative against subsequent undesirable or harmful microbic change.

The significance of this preservation of the food value of milk through lactic-acid fermentation, or souring, is readily appreciated when it is recalled that the iceman is wholly unknown in the hot, arid desert lands where the nomadic herdsman roams with his cattle and mares. The milk of his herds and flocks furnishes no inconsiderable portion of his daily food.

Soured or curdled milk has been an important and much-esteemed nutriment, even from the early days of recorded history. Abraham (Genesis XVIII, 8) placed curdled milk [1] — and a young ox — before his visitors while he stood under a tree, and

[1] The term "butter" is used, but careful studies of the original text by competent Hebrew scholars have led to the conclusion that curdled milk, not butter, is the correct translation.

they ate. Moses (Deuteronomy XXXII, 14), recounting to the children of Israel the many bounties they enjoyed, spoke of curdled milk (again the erroneous term "butter" is used) of cows and of goats, the fat of lambs and rams, of wheat and the blood of grapes.

With the passage of time and the accumulation of experience based upon observation, the descendants of these nomadic herdsmen of Asia Minor and the desert lands have learned how to hasten the souring of the milk of their flocks and herds in order to reduce the losses through putrefaction. When milk sours, the protein, known as "casein," or "cottage cheese," becomes insoluble and separates out as a clot or curd. This curd contains large numbers of very active lactic-acid microbes. A small portion of the curd is saved from the milk that has soured, and this is thrown into the freshly drawn milk. The lactic-acid microbes seed the milk at once and the process of souring proceeds rapidly. The modern custom of adding cultures of lactic-acid bacteria to milk that is to be churned is, therefore, merely a refinement of an early discovery.

The lactic-acid bacteria of the curd are a guarantee to the nomad that his milk shall sour, not

rot. Of course, he is ignorant of the fact that he is practicing bacterial inoculation and taking advantage thereby of a great natural method of food preservation, but he senses, even if he does not visualize, the magic of the process. If he is a good Mohammedan, he calls his lump of casein "the millet seed of the Prophet." He treasures it as a very precious possession. A portion of the parental casein ball is quite as essential for the newly wed as the trousseau or other marital armamentaria.

The Armenians, Turks, Arabs, Bulgars, and other nomads of the semi-arid lands of southeastern Europe, northern Africa, and southwestern Asia have thus learned to preserve the milk of their herds and flocks by the use of casein balls which contain lactic-acid bacilli. The procedure is virtually the same, irrespective of the nationality. The casein ball is thrown into the freshly drawn milk and withdrawn as soon as the souring is sufficiently advanced. The casein ball is carefully preserved against the action of sunlight and drying between immersions. The nomad has empirical knowledge of the germicidal action of the sun's rays and desiccation, even though he knows nothing about germs.

THE LACTIC-ACID BACTERIA OF THE ALIMENTARY CANAL OF MAN. The alimentary canal of the nursling becomes automatically populated with lactic-acid microbes within a very few days after birth. Strangely enough, it is not known with definiteness where these lactic-acid bacteria come from. It should be realized, however, that the normal lactic-acid bacteria found in the human alimentary canal are biologically different from those of the nomadic casein ball or curd. Notwithstanding the fact that both "the millet seed of the Prophet" and the intestinal microbes make the same chemical substance, lactic acid, from milk, the human lactic-acid bacteria would grow very poorly, indeed, in the nomadic milk pail, or goatskin, and, likewise, the lactic-acid bacteria of the casein ball would fail to grow at all in the human alimentary canal.

The nomadic microbes work part time and remain nearly quiescent between milkings. The human microbes work all the time. The whole environment of the respective kinds of lactic-acid bacteria differs, and it is not surprising to find that each respective microbe has through long ages become peculiarly adapted to its surroundings. One cannot replace the other, either within the

human body or in the milk pail. In other words, the mere fact that the two microbes produce lactic acid in milk does not imply that they are identical. It will be recalled that even the diphtheria bacillus, the typhoid bacillus, and a host of others, produce lactic acid when placed in appropriate sugar solutions. In bacteriological parlance, "things which do the same thing are not necessarily equal to each other."

The luxuriant growth of the lactic-acid bacteria in the alimentary canal of the nursling appears to be important. Statistics show beyond doubt that typhoid, cholera, dysentery, and other formidable human invaders do not develop readily in the presence of lactic acid. Cow's milk does not seem to create the same favorable conditions in the alimentary canal of the very young infant as the breast milk. Very young babies fed upon cow's milk and otherwise artificially nourished are distinctly more vulnerable to microbic infection of the alimentary canal. Pure cow's milk, however, is invaluable for the nourishment of older children, from six months to five years of age. The conclusion that the rapid and *continual* (this is important) formation of lactic acid in the alimentary canal of breast-fed infants creates conditions unfavorable

to the growth of disease-producing microbes seems incontestable.

A most important advance in the conservation of life in the first half-decade of life has been made through the vigorous and thorough inspection and care of public milk supplies. Clean, sweet milk is an important food for young children. It is far superior to dirty milk, even though the latter be soured by lactic-acid bacteria. The great decrease in dysentery and other human intestinal diseases in young children following the regulation of milk supplies is ample justification for the expenditure of the time and money it has cost. Slowly, but with sure step, man is becoming more and more the master of the microbe. With increased knowledge of the dark ways of man's hidden foes, their conquest may be confidently predicted. Each new discovery, small though it may be, is of vast importance because it affects the well-being of the population of the earth. In this respect science is unique; each advance is wholly a credit. There is no corresponding deficit. One Pasteur is worth more to the human race than a wilderness of Crœsuses.

Lactic-Acid Bacteria and Butter. Lactic-

acid bacteria play an important part in the butter industry. It has been found that cream soured with lactic-acid microbes churns more readily than sweet milk. The exact explanation is not clear. It appears to be a fact, however, that the fat droplets of cream are minute and individually surrounded by a very delicate but tenacious film of casein-like material. These films prevent the running together of the fat droplets to form the yellowish, fatty lumps which make butter. The souring process is supposed to alter the films to such a degree that they are ruptured easily by the violent agitation of the churning process. Then the fat drops escape from their prisons and gather into one solid mass.

Some kinds of microbes are supposed to add desirable flavors to the butter, such as that fanciful "new-mown hay" taste which develops when the cows are pastured in June. A variety of bacteria of this general type is known. It is customary in many dairies to add one of these to the "starter" — the lactic-acid culture — in order to combine savoriness with sourness. The preparation and sale of suitable lactic-acid bacteria for souring milk is an important industry even at the present time.

Lactic-Acid Bacteria and Sauerkraut. Sauerkraut, a well-known form of preserved cabbage, is an interesting example of two important principles applied simultaneously to the preservation of food. The cabbage is first of all cut into thin strips; then layers of this chopped cabbage, thinly sprinkled with salt (table salt), are packed tightly into casks and covered loosely to keep out insects, mice, or other undesirable intruders. The salt, in virtue of its attraction for water, withdraws much of the juice from the cabbage, and this juice, rich in sugar, ferments quite rapidly to lactic acid through the activity of the lactic-acid-producing microbes which are found on the vegetable itself. A considerable amount of foul-smelling gas escapes during the earlier part of the process. Some of the odor is due apparently to hydrogen sulphide, that odoriferous gas that occurs in some so-called "medicinal" springs. Apparently the medicinal value of the sauerkraut effluvium has been overlooked. After the active evolution of gas is over, the fermented, lactic-acid soured mass keeps well if it is covered. Access of the air frequently introduces moulds or yeasts into the product which set up secondary changes and make the sauerkraut unfit for food.

LACTIC-ACID BACTERIA AND ENSILAGE. The farmer in temperate zones has learned to store finely chopped cornstalks, or other similar food suitable for cattle, in deep air-tight cisterns called "silos." Sometimes the silo is a water-tight, concrete-lined well sunk into the ground. More commonly, the silo is a barrel-like structure of concrete or wood raised above the ground. As soon as the silo is filled, it is loosely covered. An active fermentation of the closely packed and finely chopped contents takes place, gas is liberated, and the entire mass becomes permeated with lactic acid. The gas is partly carbon dioxide (carbonic acid gas). It is heavier than air and lies as an invisible aerial blanket upon the top of the ensilage. It cannot be detected by smell or sight, but it will not support life. Occasionally a farmhand, ignorant of the presence of the gas, has been found dead in the silo. Death has resulted from suffocation, due to the lack of oxygen, rather than to an actual poisoning with the carbon dioxide itself.

The lactic-acid fermentation of the sugars in the cornstalks so alters them that they will keep for many weeks without undergoing additional change. The food value is not greatly impaired and cattle eat of the soured maize with apparent relish. En-

silage is a valuable addition to the food of cattle in the winter months. Without ensilage, the cost of keeping milk cattle would be greatly increased. It is very important to keep the retail price of wholesome milk as low as possible. Many a child's life hangs in the balance when labor and capital meet to discuss new prices for this precious commodity.

CHAPTER X

The bacteria of the alimentary canal of man — The remarkable efficiency of the intestinal incubator — The significance of the bacteria in the alimentary canal — Germ-free chicks, turtles, and guinea pigs — The importance of the bacteria in the alimentary canal of the infant — Lactic-acid bacteria and the prolongation of life.

A NORMAL, healthy adult enjoying a normal, well-balanced diet, excretes every day a mass of waste material from the alimentary canal which contains on the average about thirty trillions of bacteria. This huge number of microbes would weigh approximately two ounces. About eighty-five per cent of the substance of bacteria is moisture, which can be driven off by careful heating at the temperature of boiling water. The moisture-free substance of the daily output of intestinal microbes would still weigh about one sixth of an ounce. This number of bacteria is not readily visualized. Some idea may be gleaned, however, if it is realized that there are thirty-one trillion, five hundred and thirty-six billion seconds in one million years.

The question very naturally arises: How can the number of microbes in this alimentary waste be determined? Several methods are available. One

procedure consists of suspending a known amount of the mass uniformly in water and then making a series of dilutions until finally a small enough number of microbes is obtained to distribute uniformly over a known area upon a clean glass plate. The water in which the microbes are suspended is allowed to evaporate. The microbes are left behind and well separated. They are colored with an anilin dye to facilitate observation, and counted with the aid of the microscope. The number of microbes seen under the microscope, multiplied by the number of times the initial mass was distributed through successive known amounts of germ-free water, gives the number of microbes in the sample. This procedure is quite similar in principle to the method of counting bacteria in sewage (see page 115), where, it will be recalled, the number of microbes is very great, indeed.

Another method, equally direct, consists of the accurate determination of some constituent of the bacterial substance — for example, nitrogen — in a definite amount of the alimentary waste, after the grosser, non-microbic residue is removed. The nitrogen content of bacterial substance is quite definitely known, and it is a simple calculation to determine the weight of bacterial substance neces-

sary to furnish the amount of nitrogen found by experiment. Knowing the weight of bacterial substance, the number of bacteria is established. Several careful studies by various observers have shown that thirty trillions of bacteria is a very conservative and fair estimate of the microbes excreted from the body every day.

It might be surmised that the thirty trillions of bacteria found in the daily waste of the alimentary canal represent merely the daily intake of microbes adhering to the food. This is certainly not the case. Careful examinations of the food have shown clearly that it does not contain masses of bacteria comparable to the numbers eliminated each day; indeed, the food ordinarily contains relatively few microbes. Again, the kinds of bacteria found most abundantly in the alimentary canal are not those most conspicuous in the food. Furthermore, many microbes that grow well in the various food products outside the body fail to find conditions suitable for their development in the intestinal tract. The alimentary environment is quite unlike that of the outside world.

The unavoidable conclusion is that the greatest natural microbic incubator known to man is his own intestinal canal. The average daily output of

bacteria is equal approximately to the number of seconds in a million years, and this remarkable proliferation occurs day after day and year after year.

What is the significance of it? Is it beneficial, indifferent, or harmful? May the resident population of man's alimentary canal become a menace to health? May the multitude of germs be reduced in number or replaced in kind? Do the dietary habits of the races of mankind influence their alimentary microbes? Some of these queries can be answered, but unfortunately not all.

The explanation of the existence and the astonishing rate of multiplication of bacteria in the intestinal tract is relatively simple. The alimentary canal is a tube some thirty feet long, coiled within the body and open at each end. Food is introduced at the upper end, the mouth, and passes slowly to the other end, where the waste and useless portions are eliminated. The conditions of food, warmth, moisture, and reaction are ideal for microbic growth at all levels except the stomach and the lower portion of the large intestine. The acidity in the stomach during digestive periods is too great, and many bacteria swallowed with the food perish there. During interdigestive periods, however,

when the generation of acid ceases, bacteria may run the gauntlet of the stomach successfully; even when gastric digestion is at its height, some microbes may become enmeshed or surrounded with small masses of food particles and escape in them to the intestines.

Bacteria which pass the ordeal of the stomach acid find all the requisites for development beyond, provided they can grow in the peculiar conditions of warmth, reaction, and competition with other microbes in the intestines. However, many bacteria that do pass unscathed through the stomach fail to find suitable surroundings at lower levels of the alimentary canal. They die.

The importance of the intestinal environment as a factor in determining the growth of microbes is well shown in an experiment performed upon a fasting subject. A man abstained from all food for thirty-one days. Of course, he drank water. Naturally, there was no elimination of microbes from the alimentary canal after the first few days of the starvation period and there was no food residue to excrete. Indeed, after a few days the entire alimentary canal was thoroughly freed from food and food residues. Only the fluids naturally excreted into the tract were left. These, however, were suffi-

ciently rich in nutritive substances to keep the normal intestinal microbes alive and thriving, even though no external food was available. The number of bacteria present at this time was materially less than the normal; the kinds of bacteria were those commonly found in the well-fed subject.

This experiment shows very clearly that the alimentary canal cannot be freed from bacteria by starvation. The victim will perish long before his alimentary population is starved out.

Philosophers have long asked the question: Are the microbes which are always present in the digestive tract necessary or useful to their host? Do they promote digestion, or do they, like tapeworms, deprive the host of some of his food? Attempts to glean information upon this point have been illuminating, notwithstanding the obvious difficulties to be surmounted in obtaining them. The conclusions drawn from experiments designed to answer these queries are subject to some criticism, but on the whole they are noteworthy.

The most direct experiments have been made upon hens' eggs. These are carefully freed from adherent microbes, incubated, and hatched in germ-free incubators. The chicks which emerge are reared in a germ-free environment, fed upon

germ-free food, and provided with germ-free water to drink, and sterile air to breathe. Some of these germ-free chicks have been kept alive for many days under conditions far more exacting than those prevailing in the finest surgical operating-rooms or baby incubators. The germ-free chicks are not as interesting as chickens reared in normal microbic surroundings. Also, germ-free turtles have been reared from properly sterilized turtle eggs.

Strangely enough, the greatest difficulty encountered in the chick experiments was to procure germ-free eggs to start with. The outside of the eggshell is readily freed from bacteria. Not so, the interior. This arises from the fact that the shell is the last thing added before the egg is introduced to the outside world. Contamination with microbes occurs very commonly before the shell is formed. Even worms have been found inside the eggshell. The enclosed bacteria do not ordinarily appear to interfere with the orderly development of the chick, but at times unduly microbed eggs may prove to be a disappointment.

Another method of approaching the problem of the significance of intestinal bacteria to their host is to deliver guinea pigs by Cæsarian section, with suitable precautions against subsequent contami-

nation. The same general results were obtained as with the sterile chicks.

The net result of all these experiments, which have required so much care and the development of many ingenious devices for their completion, is to show quite clearly that the bacteria of the alimentary canal are not absolutely necessary for the well-being of their host. Very strong supportive evidence is adduced from a study of animals living well within the polar regions. It is said that the alimentary canals of the animals of the Arctic zone — polar bears, for example — are quite free from microbes, and who can deny they enjoy a normal and full existence? If they are brought to the temperate zone as captives, the usual vigorous infection and development of microbes within their alimentary canals takes place, without, however, much apparent effect upon their habits or well-being.

Intestinal microbes, therefore, although apparently not indispensable, are of necessity a part of the heritage of man and of animals. Their activities for good and for evil are to be reckoned with. Their control is one of the great problems for Science to solve. Man may turn to Nature with the assurance that the underlying principles involved will be clearly discernible.

Most adorable, and withal most helpless, is the newborn babe. It is defenseless against environment, accident, and microbe. The very essence of its existence is its food-supply. Mother Nature provided for its nourishment long before man became possessed of the necessary mental equipment to speculate upon the needs of the infant. The natural food of the nursling is breast milk. Breast milk is sterile, correctly compounded with reference to structural, energy- and heat-giving substances, and it is received by the babe unaltered by human handling. That which makes milk and many foods formidable to mankind is in no slight degree dependent upon contact with man. The nearer the producer of foods can be approximated to the consumer of foods, the smaller becomes the danger of injury by foods.

The alimentary canal of the infant is wholly free from microbes at birth, but within a very few hours thereafter invasion occurs. Various kinds of bacteria, harmless for the most part, appear in the contents of the intestinal tract. These microbes are found in the environment of the young child and they enter the body through the mouth. The first indication of a definite bacterial programme in the alimentary canal occurs about the third day of life.

By this time the babe has settled down to a fairly definite life routine. The microbic change consists essentially in a replacement of the adventitious germs of the initial invasion by large numbers of lactic-acid-producing bacteria. Lactic acid is Nature's method of preservation of food substances rich in sugars from the activity of microbes that might cause putrefaction and decay.

Breast milk is rich in that sugar known as "lactose," or "milk sugar." Lactic-acid bacteria, consequently, grow rapidly in the intestinal tract of the nursling, and it is bathed throughout and continuously with the fruits of their growth, lactic acid. They prevent in no small degree the development of putrefactive and even disease-producing microbes. Thus, they shield the infant from the undesirable or pernicious effects of undesirable germs that might otherwise develop in the intestinal canal.

In an earlier chapter, the natural preservation of milk in the tropical countries and the desert was discussed. It was shown that man has learned to induce rapid souring (lactic-acid production) by the use of "starters," which are simply lumps or balls of coagulated or curdled milk (casein) containing living lactic-acid bacteria. These microbes

grow rapidly when they are thrown into warm, freshly drawn milk. The souring they produce by their growth prevents or inhibits the development of putrefactive bacteria to a very large degree. This is important. If these putrefactive microbes were permitted to grow, they would soon make the milk wholly unfit for human consumption, or even actually poisonous.

The possibility immediately suggests itself: Why not utilize this natural preservative, lactic acid, for intestinal ailments of microbic causation? This idea has, of course, been considered. Attempts have been made to utilize it. In searching for a suitable microbe to be implanted in the alimentary canal, attention was directed to Bulgaria for two reasons. First, Bulgarians seem to enjoy a somewhat unusual span of life. Secondly, soured milk is an important part of their food. The Bulgarians use a "starter" of coagulated milk containing lactic-acid bacteria to initiate the souring process in the milk from their herds. The chain of events seems simple: longevity, lactic-acid bacteria in the intestines, in place of premature old age and putrefaction in the intestines: milk soured with lactic-acid bacteria keeps well; Bulgarians who drink this soured milk keep well. The Bulgarian sour-

milk microbe is readily obtained, freed from all other kinds of microbes, and it can be kept free from all foreign microbic entanglements by a very simple laboratory procedure. This "pure culture," as it is very appropriately called, may be kept alive and energetic for indefinite periods by repeated growth in germ-free milk.

The experiment was tried. Milk carefully soured with the Bulgarian sour-milk microbe was prescribed for those who suffer from intestinal putrefaction. The theory is sound. The beneficial lactic-acid bacillus, the active agent of "the millet seed of the Prophet," is introduced into the alimentary canal where the putrefactive parasites are at work. By its growth and generation of lactic acid, the Bulgarian microbe should make conditions unfavorable for the harmful bacteria. The model microbe, in other words, should supplant the gruesome germ, and the bacterial basis for longevity should be at once established.

Man imitated the principle underlying Nature's great method of preservation by lactic acid, but unfortunately failed utterly to follow Nature's specific directions. The Bulgarian lactic-acid bacillus grows luxuriantly in cow's milk, or mare's milk, outside the body, in the nomadic milk pail. The

human lactic-acid bacillus grows well in the alimentary canal of man. The Bulgarian bacillus fails to develop in the alimentary canal of man, however, and the human lactic-acid bacillus cannot adapt itself to the conditions imposed by the nomadic milk pail. There is no particular reason for supposing that the two kinds of lactic-acid bacteria would be able to change their environments. Otherwise, the Bulgarian bacillus should be found in the alimentary canal of the Bulgarians, and the human intestinal lactic-acid microbe should be present in the nomadic milk pail. Neither condition obtains. Pine trees do not thrive in the tropics, nor does the mahogany tree flourish in the temperate zone. Nevertheless, both pine and mahogany lumber are useful to mankind.

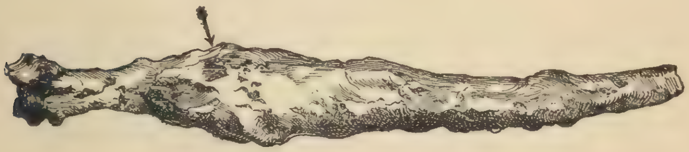
Notwithstanding the failure of the Bulgarian lactic-acid bacillus to grow in the alimentary canal, the time is coming when human intestinal lactic-acid bacteria will be used extensively in the correction of certain types of intestinal microbic disease. Science will discover and point out the conditions essential for success. Nature has been successful through countless generations of mankind.

CHAPTER XI

Man's microbic associates and antagonists — Soil bacteria and the evolution of parasitic and pathogenic bacteria — The cycle of perpetuation of parasitic bacteria — The cycle of perpetuation of pathogenic bacteria — The cycle of infection with intestinal bacteria — The cycle of infection with pathogenic bacteria of the respiratory tract — Droplet infection — Tubercle bacilli and tuberculosis — Summary of the essential factors of intestinal and respiratory infections — Plague — Contact infection.

It has been stated previously that the ancestral home of the bacteria is the upper layer of the soil. Here they are exposed frequently to unfavorable conditions of drought, heat, and cold, and of variation in the food-supply. Reason prompts the belief that some of these soil microbes must have been caught up from time to time upon the bodies of prehistoric animals, and of man when he appeared upon the earth. Here the availability of vital factors for microbic growth — food, moisture, and uniform temperature — must have contrasted very favorably with the uncertainties associated with existence in the soil. Those bacteria which could accommodate themselves to the new environment must have lost gradually their adaptability to life in the soil, and in exchange they would be forced so to distribute themselves upon the animal host that [illegible] of [illegible] descendants to other, and perhaps

Long-spined reptile from the Permian of Texas, estimated to be 25,000,000 years old



Spine from a reptile of the same kind, showing at the arrow a point of infection which has resulted in an inflammatory process (osteomyelitis) very closely akin to a modern microbic process of the same kind

THE OLDEST KNOWN EXAMPLE OF BACTERIAL INFECTION IN A VERTEBRATE

more favorable, hosts was possible. Otherwise, the microbes would have perished and thus ended the story.

The exact era in the earth's history when bacteria definitely became attached to an animal or human host — first, probably as casual visitors, later as full-fledged parasites — is unknown. The first animals to harbor microbic associates must have been living long before the age of reptiles, however, because the mighty dinosaurs clearly suffered from microbic disease. The fossilized bones of these prehistoric monsters show definite evidence of microbic infection, just such lesions as the bones of to-day exhibit. Rheumatism in an eighty-foot monster must have been a source of great discomfort had its brain development been sufficient to sense it at all. These observations would indicate that in very ancient times there was an exodus of at least some kinds of soil bacteria to the animal kingdom, where they became adapted to a parasitic existence. The earliest man whose remains have been preserved presents lesions in which tubercle bacilli are said to be demonstrable by suitable stains. The skeletons of some of these mummies show undoubted evidence of deformation by processes of bacterial origin.

THE CYCLE OF PARASITIC BACTERIA. The perpetuation of races of parasitic bacteria depends upon the serial accomplishment of three distinct steps: (1) they must reach a suitable host; (2) they must multiply in sufficient numbers upon that host to furnish descendants that (3) finally complete the cycle — namely, reach other suitable hosts. If any step in this cycle is interfered with, it is very obvious that the microbes will perish; their hosts are not immortal.

Throughout the ages many kinds of bacteria have become thus parasitized upon the various lower animals as well as man. Some live upon the skin, where escape to other hosts is very readily consummated. Some live upon the upper respiratory tract — mouth and nose; others live in various parts of the alimentary canal. The external ear, the lachrymal sac of the eye, and other parts of the body also harbor bacterial parasites. It is important to recognize the highly significant feature of this parasitic life, namely, that escape from the host is through channels opening freely to the outside world.

Parasitic bacteria do not abstract too much from the food, comfort, or well-being of their host under normal conditions; indeed, their presence is ordi-

narily unknown, unnoticed, or overlooked. It is not a characteristic part of the cycle for parasites to induce disease or infection in their patron, although many of the parasitic bacteria are "opportunists" with reference to their ability to induce infection.

In health, the intact skin, mucous membranes, and other natural barriers of the body prevent the entrance of these parasitic germs to the underlying tissues with great advantage to themselves. If, however, the continuity of the skin is broken, or if a mucous membrane becomes injured, mechanically or physiologically, a portal of entry is thereby provided for the organisms that happen to be near and they invade the wound. Accident or illness, in other words, opens a pathway which is ordinarily closed to the prospective invader. It then enters the tissues through the broken barriers, and a struggle ensues between the invader and the reluctant host. However the contest may end for the host, the parasite is almost always eliminated. It goes down to extinction. The reason is very simple. The microbe lacks the two fundamental requirements for successful tissue invasion. On the one hand, the parasite cannot of itself force an entrance through the skin or mucous membranes which

shield the underlying tissues, nor, on the other hand, can the microbe escape unaided from the tissues of the host, and thus, through its descendants, reach other hosts. The excursion into the tissues, therefore, is fatal to the microbe, irrespective of its effects in inducing infection.

The host may die or survive. If the former, the parasites which have overwhelmed their benefactor perish with him; if the host recovers and overcomes the invaders, their end is equally certain. Parasitic bacteria, in other words, which invade their host, cannot escape the consequences of their unpreparedness for a pathogenic existence.

An excellent example of a parasitic microbe is that one known as *Staphylococcus pyogenes aureus*, which occurs very generally upon the skin and mucous membranes. Ordinarily it is a harmless and, so far as known, useless resident on normal, healthy man. It is, however, an "infection opportunist," unable of itself to penetrate through the skin to underlying tissues, but endowed with the ability to cause damage in the tissues. A scratch, bruise, or rough-edged collar, or some other accident, may cause minute breaks in the continuity of the integument, and the *Staphylococci* thereby may gain an entrance. A boil, or carbuncle, may result.

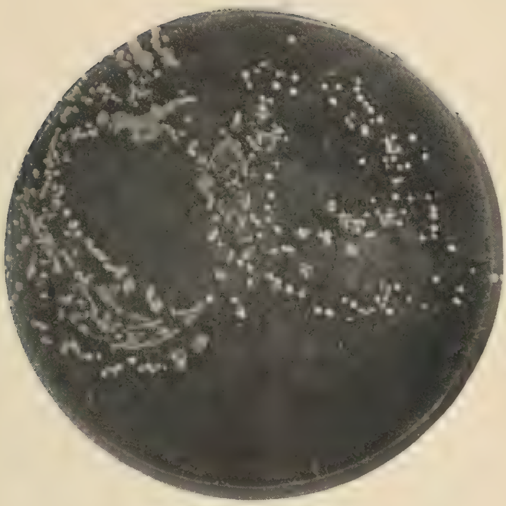
The boil may subside of itself, which indicates that the defenses of the host exceed the offensive power of the microbe. The boil may become recurrent, thus indicating a struggle on more equal terms between host and invader. Sometimes the morbid process is very malignant, and death of the host follows. In any event, the microbe perishes. Epidemics of boils are unknown. The majority of *Staphylococci* pass through the normal channels of parasitism, and their descendants perpetuate themselves successively in other hosts.

THE CYCLE OF PATHOGENIC BACTERIA. Out of the class of parasitic bacteria which have adapted themselves to an existence upon the bodies or open body cavities of special hosts, man or animal, there has arisen a smaller group of bacteria, comparatively few in numbers, but extraordinarily formidable in that their activities are directly in opposition, or partial opposition, to those of man or animals. Like the parasitic bacteria, the pathogenic microbes require a living host for their perpetuation. That is to say, they must reach a suitable host, multiply thereon, and escape to successive suitable hosts in order to perpetuate themselves. The pathogenic bacteria, however, unlike the parasitic mi-

crobes, can of themselves force an entrance into the tissues of the host, and escape from the tissues when multiplication therein has taken place. They are finished criminals. Every step of their pernicious cycle is carefully worked out. Unlike the amateurish parasite, which enters whenever and wherever an opening presents itself, the progressively pathogenic microbe selects a definite point in the body of the host for its initial skirmish. With the exception of infections carried by direct contact or by insects, this is usually the digestive or the respiratory tract. The general features of contagion will be clearer if a few types of infection are cited.

THE CYCLE OF PATHOGENIC INTESTINAL BACTERIA. Typhoid fever is a characteristic example of the alimentary route of infection. Paratyphoid fever, bacillary dysentery, and Asiatic cholera are other well-known diseases which agree with typhoid in the general features involved, but differ among themselves somewhat in details.

Typhoid bacilli reach the prospective host and victim through water, milk, food, flies, fingers, or filth, infected directly or indirectly from a previous case of typhoid fever. The contaminated food or



THE TRAIL OF THE TYPHOID FLY

A fly was allowed to feed upon a culture of typhoid bacteria. Then it was forced to walk over the surface of some sterile gelatin. Each white spot, containing hundreds of thousands of typhoid bacteria, represents one footprint

water runs the gauntlet of the stomach acidity, and eventually live typhoid bacteria may reach the small intestine of man. There is a peculiar kind of tissue in the small intestine known as "Peyer's patches." This tissue is vulnerable to the typhoid bacillus, provided the latter comes in sufficient numbers and possesses the requisite aggressiveness to overwhelm the resistance which the tissue of Peyer's patches offers to invasion. Inasmuch as the ordinary intestinal parasitic bacteria fail to force a passage through the patches, this resistance must be considerable.

There is no method of determining the exact conditions which must exist between host and typhoid bacillus whereby penetration does or does not take place. One onslaught may result in failure while repeated attempts may be successful eventually. There is no doubt that many individuals escape infection, even though the enemy appear at the gates of all.

If the attack upon the host's frontier at the Peyer's patches succeeds, the typhoid bacilli pass into the tissues and a new phase of the struggle begins. The blood of man and animals possesses peculiar powers in virtue of which moderate numbers of many kinds of bacteria are destroyed out of

hand. Also, certain kinds of cells in the blood and among the tissues are able to pick up and digest moderate numbers of predatory microbes. If the germ-destroying powers of the blood and the scavenger cells of the blood and of the tissues suffice to destroy those invaders which have forced an entrance through the frontier cells of Peyer's patches, the battle is lost for the time being to the invader, and little or no reaction of the host indicates that a victory has been won. A second invasion may succeed even if the first failed, provided the second attack is massive enough to overcome the primary and secondary defense.

If all the defenses fail, the microbe multiplies within the tissues as an actual invader and a new phase of the conflict between man and microbe has begun. On the part of the host, latent offensive mechanisms are roused into specific activity. All of the ordinary, normal preparedness having proved inadequate to turn the tide against the invader, various groups of cells of the body prepare and ship, by way of that great highway of internal communication, the blood-stream, specially designed and prepared munitions which shall be specifically harmful to the typhoid bacillus. They are quite ineffective against other intestinal invaders,

as dysentery or cholera germs. If the specific offensive of the host is decisive, the typhoid bacillus is overwhelmed and the attack is at an end. Peace reigns within the frontiers of the body, and *post-bellum* recovery commences. The lesson is learned, however, and forever after the individual who has passed successfully through a siege of typhoid fever is thoroughly prepared to resist a subsequent invasion. Typhoid fever rarely recurs in the same individual.

During the time when the human machine is elaborating a specific attack upon the invading microbe, the microbe, like a cunning bandit, is providing for a continuance of its offensive in another, succeeding host. Before the offensive of the host becomes too powerful, the typhoid bacilli, greatly and even enormously increased in numbers by their rapid growth in the victim, have already begun to escape to new and non-immune hosts. They break out from the tissues through the ulcerated Peyer's patches, the gall bladder, and many times from the urinary bladder as well, and escape with the intestinal contents, and less commonly the urine, to the outside world. Then they find their way into water-supplies that are used for drinking and domestic purposes; they may get into milk or food

from infected fingers; they may be distributed by flies, by shellfish drawn from infected estuaries, and in many other ways.

Typhoid fever in a community means a criminal short circuit between the intestinal waste of a patient and the mouths of innocent fellow men. It is needless to point out the obvious remedy — destroy the infective discharges at their source.

Sometimes the struggle between patient and microbe results in a drawn battle — the patient becomes immune to further illness, although the microbes remain comfortably located in the gall bladder or possibly other parts of the alimentary canal, quite sheltered from the external world, but freely in communication with it through the intestinal evacuations. Such unfortunate and usually unsuspected harborers of typhoid bacilli are called, very appropriately, "typhoid carriers." They may excrete typhoid bacilli for many years. Other progressively pathogenic bacteria may be likewise distributed. The bacteria carrier is an important source of infection in a community.

The general phenomena common to all intestinal infections are exemplified in typhoid fever. The prevention of all contagious or infectious intestinal diseases, be it typhoid, cholera, dysentery, or any

other, is the same. Destroy infected intestinal and urinary discharges.

THE CYCLE OF PATHOGENIC BACTERIA OF THE RESPIRATORY TRACT. Another great class of progressively pathogenic infections are those involving the respiratory system. Many bacteria gain entrance to the tissues of the body through that vast system of channels which make up the respiratory tree. These open to the outside through the trachea and the mouth. They enter the lungs through an ever-narrowing and constantly branching series of tubes — bronchi and bronchioles, as they are called — until they finally terminate in hundreds, even thousands, of minute terminal twigs, each supplying and removing air from a group of tiny, saclike enlargements known as "alveoli." The combined surface area of these alveoli has been estimated to be over ninety square yards.

The lining of the air tubes for no inconsiderable distance from the outside is a layer of cells, each provided with one or more short, delicate, threadlike appendages known as "cilia." These move rhythmically in such a manner that the forceful thrust is upward and therefore outward. The re-

covery for the next movement is less vigorous. The gross effect of this mass of contractile, whiplike hairs is to prevent solid particles from penetrating deeply into the air passages. If such manage to reach the level of the vibrating cilia, they are pushed upward and outward quite as if they were thrust upon an escalator. Bacteria are thus usually thrust out and prevented from entering the deeper and more vulnerable terminals. Cold paralyzes, or at least decreases markedly, the energetic ciliary movements. Cold may be, and not improbably is, therefore, a factor in promoting infection of the deepest recesses of the lungs.

Tuberculosis is an important infectious disease which commonly localizes in the lungs. The germ that causes the infection is very appropriately named the "Tubercle Bacillus." This microbe, leaving the lungs of a careless consumptive, is coughed upward and outward with hundreds or even thousands of its fellows, and scattered in the air about.

Droplets of infected sputum, very minute but large in comparison with the microbes, may remain suspended in the air for an indefinite period. Currents of air may waft these infected droplets for many feet or yards. In theaters, cars, and crowded

schools the chances of inhaling these infected droplets are very numerous.

Droplet Infection. The question may very fairly be asked, How does one know that infected droplets of sputum may be carried for considerable distances through the air? Fortunately, a crucial experiment has been performed which exactly fulfills the essential conditions under discussion.

A harmless microbe, known as *Bacillus prodigiosus*, was selected as the test germ. *Bacillus prodigiosus* grows readily upon many organic substances, and it is sometimes found in the air. In the early part of the nineteenth century, the city of Padua was greatly excited by the appearance of blood-red spots which appeared upon the bread exposed on the altar in one of the churches. This was called the "miracle of the Bleeding Host" by the peasants, but it was soon discovered that the red colorations were due to growths of *Bacillus prodigiosus*, which was identified by an analysis of the air.

To return to the experiment: A small amount of *Bacillus prodigiosus* is introduced into the mouth of a demonstrator, who takes his stand at one end of a room previously prepared for the experiment by exposing at various levels and places dishes of

sterile gelatin. If the room is shown by previous experiments to contain no microbes capable of growing as red-colored spots on the gelatin, it may be assumed that the appearance of red growths on the gelatin plates, after a period of hours, represents the microbes that have been actually transmitted through the air when the experimenter talks, coughs, or sneezes. The results of many trials have shown quite definitely that microbes are actually expelled from the mouth of an individual. Also, they may pass through the air and lodge in the nose or mouth of another individual.

In the experiments with *Bacillus prodigiosus*, growths were obtained in the uttermost corners of the room, about forty feet distant, and, in one instance, the microbes actually were driven by a gentle current of wind from the experimenter's room through a hall and up a flight of several stairs!

Quicker results may be obtained by a chemical method, if only the actual transmission of droplets is to be demonstrated. Sheets are dipped in a weak solution of bicarbonate of soda and hung around the room at various points. The experimenter places a small amount of a colorless solution of an anilin dye, known as "phenolphthalein," in his

mouth and then talks or sneezes or coughs. The dye turns a bright red when it reaches the alkaline soda solution. Very soon after the start of the experiment, pink or red spots appear on the sheets, sooner and larger very near the speaker, smaller and later in the far corners. Inasmuch as phenolphthalein is never a constituent of the air, it is very clear that some of it must have traversed the air space between the speaker's mouth and the point of lodgment on the sheets. The evidence that droplets may pass from the mouth (or nose) to points some distance away seems very conclusive.

Tubercle bacilli, or the microbes of any infection of the respiratory tract — whooping-cough, pneumonia, scarlet fever, measles, "flu" (influenza or la grippe), colds — may thus be coughed out from one patient and transmitted through droplets to other prospective victims. The tubercle bacillus is fairly illustrative of the process, although it is a chronic, long-drawn-out disease in contrast to flu, whooping-cough, and other well-known acute respiratory infections.

To return, then, to the tubercle bacillus, which has been coughed or sneezed into the air from the depths of the lungs of a careless consumptive — the

infected droplets either gain access directly to the nose or mouth of a near-by, innocent bystander, or the bacilli may remain in the air for a period of time and then be drawn into the respiratory tract of the victim. In rooms, the microbes may remain on the floor, if they are not inhaled by a patient, and, later on, be raised with the floor dust by sweeping or other disturbance, and then gain access to a man.

A baby creeping over such an infected floor may be quite as readily invaded by tubercle bacilli as may guinea pigs, which have been deliberately exposed to floor dust with fatal results.

If the tubercle bacilli do gain access to a man through infected droplets or dust, they may pass through the series of barriers that are designed to keep microbes from the depths of the respiratory tract. These are: the moist, tortuous course of the nasal mucous membrane, the upward thrust of the hairlike processes of the lining cells of the deeper bronchi, and probably also the projectile-like effect of coughing which tends to remove irritant or foreign particles from the respiratory tract. Also, migratory cells and mucous cells protect the deeper parts of the respiratory tract by surrounding or engulfing foreign substances.

Let it be assumed that the tubercle bacilli have

run the gauntlet of this entire line of defense and have reached the minute, saclike enlargements of the terminal bronchioles. The microbes then become attached to the wall of the alveoli and grow there. Gradually a more or less spherical mass of cells forms, layer after layer, becoming larger and always larger, in a vain attempt to hem in and restrain the growth of the invaders. This is called, very appropriately, a "tubercle." Sometimes the body succeeds in throwing enough of these cells and their products around the nidus of microbes to keep them in check, or even kill them. Sometimes the offensive of the body suffices merely to keep the microbes from growing. They remain alive and latent, but ready to start anew their campaign of conquest if the effectiveness of the barrier is overcome by excesses of various kinds, colds, intermittent infection, or other disabling agency.

In this event, the onward march of the microbes is again in evidence, and the ever-widening and ever-weakening lines of defensive cells of the lungs thrown around the enemy in their midst become so drawn out that the older and more central cells lose contact with the blood. Nutrition stops, the cells die, and then a cavity has formed in the lung tissue. The area of softening increases in diameter, and a

time comes when the edge of the area reaches a bronchus in communication with the outside world. The cheesy contents of the cavity empty into the channel communicating without, and the irritation of the semi-fluid substances incites coughing. The tuberculous sputum raised by the effort of coughing (which is repeated for days at a time) contains a multitude of tubercle bacilli free from the body and ready to be wafted to other hosts. The cycle is complete. The microbes have reached a host; they have passed through a suitable portal of entry to these tissues (alveoli); they have penetrated, or been forced, into the tissues; they have grown there and multiplied abundantly; and, finally, they have reached a channel in free communication with the outside world, the respiratory tract.

There is much evidence to show that fingers, eating-utensils, and other agencies whose destination is, or may be, the mouth, may carry tubercle bacilli from consumptive to prospective victim.

Sometimes the tubercle bacilli, growing rapidly in the lungs, erode through a blood-vessel that may happen to be in the mass of the tubercle. The microbes then enter the blood-stream, and are carried to all parts of the body. Defenses are lacking at the new points of invasion and a rapid development of

tubercles occurs which soon overwhelms the patient. Acute miliary tuberculosis, as this process is sometimes called, does not resemble the ordinary clinical picture of tuberculosis; it strongly resembles the course of a case of typhoid fever. This form of tuberculosis is very fatal to the tubercle bacilli. The patient dies too soon, as a rule, for the microbe to establish a connection with the outside world. They succumb with their victim. None survive to reach other hosts. Miliary tuberculosis is not contagious.

Tubercle bacilli are sometimes carried from tuberculous cattle to man through their milk. The portal of entry to the tissues is through the alimentary canal in such cases, and the disease is not transmissible from man to man so far as available information indicates. This form of tuberculosis — Bovine tuberculosis — is therefore not a matter of concern to the student of epidemics.

The general phenomena of infection of the respiratory tract by microbes that are "progressively pathogenic" — that is, that cause disease from man to man — are, on the whole, much like tuberculosis, except that the process is usually acute and runs its course in days where the tubercle bacillus requires months or even years. The microbes in-

fect some part of the respiratory tract and reach and leave the patient through the bronchi, mouth, and nose. The spread of these diseases, as whooping-cough, "flu," poliomyelitis, and other well-known infections of the same general kind, is presumably for the most part droplet infection. The method of transmission is by coughing, by sneezing, or talking with force enough to expel droplets of infected sputum from the mouth or nose.

Tramcars, theaters, and places of public gathering are admirable clearing-houses for the exchange of current microbes. Except in times of epidemics, however, the danger of general infection is slight — much less than that of the ordinary hazards of life.

The prevention of all the respiratory infectious diseases is quite simple — infected droplets must not be permitted to escape from the mouth or nose. Also infected linen, dishes, chewing-gum, or pencils must not be permitted to pass from patient to prospective victim without sterilization.

Summary of Intestinal and Respiratory Infections. There is, then, a group of microbes that belongs to the class of pathogenic bacteria. These are of two principal types: those that infect the alimentary canal and those that infect the

respiratory tract. Both enter the body through the mouth (or through the nose, in respiratory infections). The former leave the body through the discharges from the intestines, or rarely by the urine; the latter leave the body through the mouth or nose.

Prevention of infection is therefore readily understood, in principle at least. Destroy infected intestinal discharges, or destroy infected mouth and nose discharges.

INSECT-BORNE INFECTION: PLAGUE. One more type of infection deserves mention — plague. Plague usually is carried from an animal, as the rat, marmot, or ground squirrel, to man through the flea. Sometimes plague appears as an infection of the respiratory tract. This type is known as "pneumonic plague." It is highly contagious, and doctors and nurses who care for patients are running greater danger of infection and death than from any other known disease. The microbes in pneumonic plague are coughed up in large numbers by the patients, and the disease is extremely severe, if not fatal.

The ordinary type, known as "Bubonic plague" because the glands in the thighs and armpits be-

come greatly swollen (buboes), requires a peculiar and apparently wholly accidental means of transportation from rat to rat, and from rat to man.

The plague microbe, known technically as *Bacillus pestis*, does not belong to the group of the progressively pathogenic bacteria thus far described (exception is made of pneumonic plague). The germs have not developed a method of entering the tissues of themselves, nor can they escape from the tissues of themselves to infect other men or animals. With the exception of the pneumonic type (which, of course, is a droplet infection, as "flu," or whooping-cough), the plague bacteria require the help of the flea to carry them from host to host. The cycle of infection is as follows:

A flea bites a rat or man who is suffering from plague. The microbes which are locked up in the body of the patient, but circulating in the blood-stream, are taken up with the blood the flea requires for its food. The germs enter the stomach of the flea with the blood and remain alive there for some time. It is not definitely known that they multiply in the flea's stomach. When the flea is ready to bite again, it first empties its alimentary canal. The plague bacilli are deposited upon the skin. Then the insect bites. An intense itching fol-

lows the bite. The attempt to relieve the discomfort by vigorous rubbing or scratching actually forces the microbes through the skin into the underlying tissues. Possibly, but not necessarily, the germs may actually enter through the minute puncture left by the bite.

Many students of the plague believe that the rat flea, which finds the rat an acceptable domicile, is reluctant to leave a rat for a man. Fleas, however, are apparently induced by some unknown premonition to leave their dying host, be it rat or man, and seek one that is normal. Therefore, presumably after a last feeding upon the blood of the dying host, the insect leaves the victim even as rats are said to desert a sinking ship, and jumps to a new one. If no rat is in sight and a man is accessible, the rat flea will take to the man rather than perish. The first thing the flea does after moving into the new environment is to draw a full meal from the blood-stream of the host. The customary emptying of intestines already infected with plague bacilli precedes the act of biting. The itching resulting from the bite causes the microbes to be forced into the underlying tissues, and infection sets in. This, in brief, is the usual course of infection with the plague microbe. It differs from the

intestinal and respiratory infections markedly, in that the plague microbe does not seem to reach a new host through droplets or infected food, water, or utensils. All depends upon the flea.

Contact Infection. Diseases which are transmitted by contact infection require no explanation of their cycle from victim to victim. The inoculation of a sound person by a diseased one is as direct as the inoculation of sterile beef tea from a culture of microbes in another tube of beef tea. Many times, however, the victim does not realize that infection by direct inoculation is taking place.

CHAPTER XII

Immunity, susceptibility, and resistance to infection — The origin of microbiphobia — Susceptibility and resistance to microbic invasion — The general phenomena of resistance to bacterial infection — Natural, individual, and acquired immunity — Defenses of the body against infection with parasitic bacteria — The offense of the body against invasion by progressively pathogenic bacteria — The production and use of antitoxin — Theories of immunity and their relation to parasitic and pathogenic bacteria.

The prevailing conception of a microbe seems to be that of an unseen foe which generates pestilence and death. This is not surprising. Unknown agencies that oppose man are of greater concern to him than those that operate to his advantage. Sacred history describes a plague that appeared among the Philistines when they captured the Ark of the Lord. Their leaders were ordered by the priests to restore the Ark to the Children of Israel and to make "five golden emerods and five golden mice" as a peace offering (1 Samuel, v, 11; vi, 4). It is believed that this pestilence was Bubonic plague, a disease characterized by swelling of certain glands (buboes, emerods), and transmitted from mice (rats) to man by fleas. The terrible isolation of the leper also is set forth most vividly: "And the leper in whom the plague [disease] is, his clothes shall be

rent, and his head bare, and he shall put a covering upon his upper lip, and shall cry, 'Unclean, Unclean.'" (Leviticus, XIII, 45.)

Plague invaded England in 1348 and in 1665. In London alone one hundred thousand of the inhabitants, it is claimed, perished in the earlier epidemic, and seventy thousand in the later one. Contemporary writers have left an imperishable record of the terror and suffering entailed by these great microbic tragedies, the effects of which on the social and religious life of the people were profound and far-reaching. Many other extensive outbreaks of disease have been recorded, extending even to the present time. It is self-evident that the microbe occupies a prominent place among the forces of nature operating destructively against mankind.

Whenever the ruins of a lost and forgotten civilization are discovered, and there seems to be no tangible explanation for its decline and disappearance, the microbe is advanced as a plausible scapegoat. Calm reasoning dictates, however, that the natural outcome of the struggle between mankind and microbe has always favored man and restrained the microbe. Otherwise, mankind would have perished from off the earth. It is highly improbable that any microbe has ever wiped out an entire nation.

Previous chapters give in brief review some of the more prominent fields of human activity in which the microbe plays an important or even an indispensable part. The sum total of microbic participation in the processes of life is overwhelmingly on the side of beneficence. Indeed, all the notoriety that attaches to the microbes arises from the interference of a small but extremely formidable group of bacteria whose activities are in opposition to those of man. It was ever thus. "The evil that men do lives after them; the good is oft interred with their bones."

The phenomena of microbic infection, the resistance of man to infection, the susceptibility of man to infection, the balances between man and microbe which prevent the overwhelming of the human race by its bacterial foes, and the gradual conquest by man of the disease-producing germs, constitute a chapter in the history of civilization which is yet to be completed.

The intricate, reciprocal relations which exist between man and microbe bring into sharp relief a biological struggle between a host and an invader, of such amazing complexity that its solution will require a knowledge of the fundamentals of life itself. Nevertheless, considerable progress has been made

and many facts have been established. The master minds of each generation are working toward its solution. The conquest of the disease-producing microbe is slowly advancing. The final exposition must await the perfection of knowledge in the fields of chemistry, physics, and physiology.

Some of the outstanding features of microbic infection are readily distinguishable. It is generally known, for example, that man is quite free from the epizoötic diseases of the animals he has domesticated; also, it is well established that animals do not contract the contagious diseases of mankind. Thus, fowl do not sicken with Asiatic cholera nor do men acquire fowl cholera. Typhoid fever and whooping-cough are unknown among the animal species, and hog cholera and Texas fever of cattle are not recorded in the list of human diseases. It is true that a very few diseases of animals, as, for example, Malta fever (of goats), rabies, bovine tuberculosis, and glanders, may be transmissible from animal to man. It should be observed, however, that these infections are not, as a rule, retransmitted from one man to another man. In other words, they are not contagious among mankind.

Closely related animal species manifest a puzzling difference in susceptibility to one and the

same microbe. Algerian sheep, so the claim is made, may be pastured with impunity on farms so thoroughly infected with anthrax bacilli as to be deadly for the common breeds of sheep. Field mice are said not to succumb readily to the glanders bacillus, a deadly infectant of house mice.

Again, many individuals among an otherwise susceptible race exhibit striking resistance to specific infection. Thus, certain members of a family may escape that highly contagious disease scarlet fever, even though brothers and sisters fall victims of this dread malady. Many members of a community escape typhoid fever, even though the virus be generally disseminated through a contaminated water-supply. A very small number of cases of meningitis or of infantile paralysis appear in a community where the majority, the great majority, of people escape. On the contrary, a world-wide epidemic — a pandemic — of la grippe, or "flu," claims its thousands of victims in the exposed community.

Recovery from certain types of infections, as boils or contaminated wounds, does not seem to confer upon the patient freedom from subsequent infection; indeed, one crop of boils rather predisposes toward a second infliction. On the other hand, con-

valescence from the great majority of those diseases designated "contagious," as, typhoid fever, smallpox, measles, whooping-cough, and many others, carries with it relative security against a second infection of the same kind.

Specific names have been applied to these various states of natural and acquired resistance to microbic infection.

1. When the members of a race or species are observed to be resistant to infection with a specific germ, they are said to be "naturally immune." This immunity, or non-susceptibility to specific infection, is designated "inherited," or "congenital, natural immunity." Thus, man is naturally immune to hog cholera.

2. If some, but by no means all, of a race or species exhibit refractoriness to infection with a specific microbe, those members which resist infection are said to enjoy "individual natural immunity." The condition is frequently referred to as "individual immunity." Thus, some but not all members of a family may fail to become ill with scarlet fever.

3. That resistance to reinfection which follows recovery from illness induced by a specific microbe is very properly designated "acquired immunity," in contradistinction to that natural immunity

which confers lifelong exemption from infection with a given virus. Thus, recovery from an attack of typhoid fever usually renders the convalescent immune to subsequent attacks.

These striking relations between man and different kinds of bacteria could not fail to stimulate investigation; indeed, what field of study could offer greater prospects for service to humanity than that which should unfold the reasons for these empirically determined facts? What factors predetermine the resistance of man to many bacteria which are formidable to domestic animals? Why should recovery from infection with certain kinds of microbes result in freedom from a second infection by them? And what is lacking in the human defense to those bacteria which apparently find a second invasion somewhat more readily accomplished than the first? In other words, why should mankind be naturally exempt from infection with one kind of microbe, and acquire exemption from reinfection with a second type of microbe, and appear to become predisposed to reinfection with a third type of microbe? Is it possible to create resistance to microbes by any safe procedure and thus reduce the hazard of microbic disease?

The bacteria that have become adapted to man

as their specific host — humanized microbes, as it were — may be divided into two rather general and distinct types for purposes of discussion. One type includes the parasitic bacteria. These grow normally upon the surface of the body, or in cavities of the body which open freely to the surface. The escape of the parasitic microbes to other suitable hosts (the perpetuation of the race, in other words) seems to depend very largely upon this exposed position. The intact skin and mucous membranes prevent their entrance to, and egress from, underlying tissues. Excursion into the tissues is fatal to the perpetuation of the race.

The other type comprises those microbes that cause progressive disease from man to man. Their multiplication, which insures descendants to perpetuate the race, takes place to no inconsiderable degree within the tissues of the body. It is quite obvious that these pathogenic bacteria must possess the inherent ability to force a passage through and from barriers which resist the penetration of parasitic microbes. In other words, parasitic bacteria enter the tissues of the body only at points where injury or accident weaken the normal barriers. Progressively pathogenic bacteria, on the contrary, can of themselves pass in and out through

these same normal barriers. Also — and this is important — the progressively pathogenic bacteria enter at definite points, usually within the respiratory or digestive tracts.

The body does not tolerate bacterial growth in the tissues. It must be prepared to meet two kinds of microbic invasion. One kind, induced by the accidental invasion of parasitic germs, may occur at any part of the body. A scratch on the tip of the nose, or the tips of the toes, affords an equal opportunity for a *parasitic* microbe to leave the surface of the body, where it normally belongs, and become an actual invader. The *progressively pathogenic* germs are more fastidious in their choice of a portal of entry. Typhoid bacilli, for example, cannot enter to underlying tissues through the skin or through the lungs; they infect through the intestines. Influenza microbes, on the contrary, are apparently unable to attack through the intestines or the skin, but penetrate through the respiratory tract.

Nature has devised a defense against this threat of invasion by parasitic microbes, which may take place at any time, and may occur at any point.

THE DEFENSE OF THE BODY AGAINST PARASITIC BACTERIA. In the circulating blood, which nour-

ishes every organ and tissue of the human body, there is a multitude of wandering, independent cells known as white blood-cells, or "leucocytes." They are not as numerous as the red blood corpuscles, which number about five million in each drop of blood. There are approximately seventy-five hundred leucocytes to every five million red blood-cells. These leucocytes are the armed patrol which the body maintains to insure the freedom of the blood-channels from predatory microbes. The white blood-cells are police, judge, jury, and jail, all in one. They arrest, engulf, digest, and destroy the criminal germs. If microbic outlaws enter the blood-stream, these leucocytes march upon and annihilate them. If the germs enter the tissues, the white blood-cells swarm after them, and congregate around the alien horde in ever-increasing numbers. The felonous host is gradually surrounded, engulfed, and destroyed. The field of combat becomes strewn with the dead and dying corpses of microbes and leucocytes alike. In an ordinary infection, as a boil, the entire mass becomes partially softened and decomposed — necrotic, as the pathologists say — and eventually forms that gruesome mass known as pus. When the boil is "ripe" — that is, when the outlaw microbes are fairly under control — the

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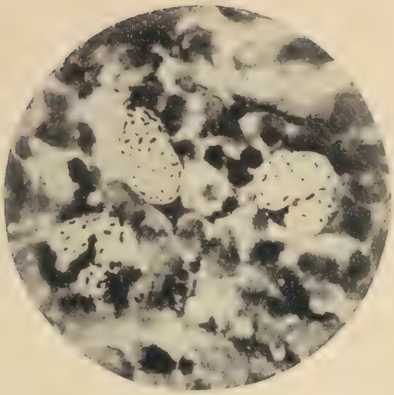

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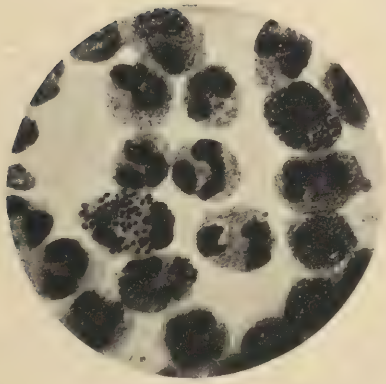

LEUCOCYTES, OR WHITE BLOOD-CELLS, FILLED WITH BACTERIA

1. Secretion from nose during acute inflammation
2. Fluid from spinal canal in cerebrospinal meningitis

evacuation of the pus usually leads to complete healing. Sometimes the outlaw germs prevail and cause widespread destruction in the body, or even death. Occasionally, the microbes settle down in a joint, or the heart, and keep up a languid opposition to the efforts of the body to dislodge them. Usually, however, the leucocytes prevail and restore order. Infections with the parasitic bacteria rarely or never reach beyond the body of their first victim. Epidemics of boils, of infected joints, or involved hearts, practically never occur from man to man.

Recovery from invasion with parasitic bacteria does not result in immunity to subsequent invasion with the same kind of microbe. The leucocytes that are veterans of one insurrection do not ordinarily survive long enough to participate in another. They are short-lived. They leave no descendants.

The salient facts relating to the leucocyte defense of the body against sporadic invasion of the tissues of the body by parasitic microbes were discovered by a noted Russian scientist, Metchnikoff. He wove these facts into a theory which is called the "Cellular" or "Phagocytic (scavenger cell) Theory of Immunity." His experiments and those of his colleagues and followers explain very satis-

factorily the defensive mechanism which shields the body from the baneful possibilities of parasitic invasion. This theory is not in reality a theory of immunity, however: it is a theory of body defense. Immunity to parasitic invasion has not been demonstrated. Attempts to reconcile the phenomena of the leucocytic defense of the body with that true immunity that follows recovery from infection with microbes of the progressively pathogenic type (pathogenic, or contagious bacteria as the typhoid bacillus, for example) have signally failed. The two types of infection are quite unlike.

THE OFFENSE OF THE BODY AGAINST PROGRESSIVELY PATHOGENIC BACTERIA. The bacteria that cause contagious disease are not normal residents of the sound body. They are alien invaders. They can overwhelm barriers of the human frame which keep out the normal microbic residents. They must, however, each after its kind, penetrate through definite points to the underlying tissues. To combat invasion by pathogenic microbes, the entire body enters upon a state of war. New weapons are forged. The effects of invasion are widespread and affect the entire human mechanism. The struggle begins when the prospective alien in-

vader reaches the point where the frontier must be crossed. When a typhoid bacillus, for example, is brought to the mouth of a non-immune individual by contaminated food, fingers, flies, water, or milk, it must first of all run the gauntlet of the stomach acidity. If it succeeds, it must pass to the Peyer's patches in the small intestine. Here it meets the resistance of the intestinal wall, which must be overcome before the microbe can enter the tissue within. The tissues in turn are bathed with blood, which possesses to a limited degree the power of dissolving and killing predatory microbes. If this obstacle is surmounted, the body for the time being is at the mercy of the microbe. Any or all of these defenses may hold and destroy the typhoid bacillus. If such is the case, the invasion fails. There is neither sign nor symptom, however, to indicate that a victory has been won.

If the microbe overwhelms these defenses, it grows rapidly within the body, for a time without detectable opposition. Soon, however, a new phase in the struggle is noticeable. Defensive measures having failed (leucocytes do not seem to play a prominent part in the process), the body mobilizes latent offensive weapons, and starts a definite campaign against the enemy within the gates. New sub-

stances appear within the blood-stream, which are specifically designed to destroy the alien foe. It is important to realize that these specific substances are ineffective against any other microbe. The specific munitions accumulate in great and ever greater amounts, until a great surplus is built up. This finally exceeds the actual requirement to destroy the germs. In eighty to ninety per cent of cases, the tide is turned and the foe is dislodged or destroyed.

During the period between the failure of the defenses, and the perfection of the offensive, however, the typhoid bacilli reënter the intestinal tract in large numbers and escape with the intestinal waste to the outside and thus reëstablish communications leading to other prospective victims. In this manner the perpetuation of the race is provided for.

To return to the specific offensive of the body: the blood of the patient convalescing from typhoid fever contains new substances which will dissolve the microbes, as well as substances which will cause them to clump together. These specifically designed products of offense against specific microbes have received names. Those that dissolve bacteria are named "lysins" (dissolvers), or "bacteriolysins" (bacteria dissolvers). Those that cause microbes to flock together, or clump, are designated

as "agglutinins" (agglutinators). All of these substances that are newly formed and specific for individual microbes are called in general "anti-bodies," that is to say, substances that are antagonistic to specific germs.

Toxin and Antitoxin. Sometimes the microbe does not penetrate through, but grows upon a mucous membrane, and secretes a violent poison (toxin) which enters the blood and weakens or destroys the cells of the body. Such a microbe is the diphtheria bacillus. If the flood of poison is not too deadly, the tissues gradually form an antipoison, or antitoxin, which neutralizes the poison or toxin.

Carefully standardized antitoxin for the treatment of diphtheria is one of the great discoveries in Bacteriology. The method of preparation of antitoxin deserves passing mention. Diphtheria toxin (poison) — see page 70 — is injected into horses in gradually increasing, but of course relatively harmless, doses. The tissues of the horse respond by the production of the specific neutralizing substance, "antitoxin." When this is present in relatively large amount, the horse is bled, the serum is separated from the red blood-cells and leucocytes, and carefully sterilized. This horse serum, containing

the antidote for the poison of the diphtheria microbe, is called "diphtheria antitoxic serum." It will neutralize the diphtheria toxin in a case of human diphtheria.

Attempts to produce serums for many of the progressively pathogenic bacteria have been unsuccessful so far. Recovery from infection with these progressively pathogenic bacteria, so experience indicates, leaves the patient immune, or nearly so, to a subsequent invasion with the *same* microbe. The chemical basis for the resistance to reinfection rests in the persistence of the various anti-bodies just described in the blood-stream. They may be identified there for years after the infection has subsided.

A German scientist, Ehrlich, together with his colleagues and followers, studied the substances found in the circulating blood as a result of recovery from infection with the progressively pathogenic bacteria, and showed quite clearly how they are related to the immunity of the individual to reinfection. The theory, known as the "Humoral" or "Ehrlich Theory of Immunity," has been violently opposed by the partisans of the "Metchnikoff" or "Cellular Theory of Immunity." As frequently happens, neither theory is wholly correct nor wholly incorrect in its claims.

CHAPTER XIII

Balanced parasitism — The importance of balances in nature — The effects of unbalance — Disturbed balances by the introduction of alien species — The eradication of alien species and the reëstablishment of balance — The nature of the microbic balance with mankind — The complexity of the microbic balance; meningococcus strains and meningococcus balances — The importance of serologic strains among microbes and their effects upon the man-microbic complex — Summary and conclusion — The dawn and development of microbiphilia.

BALANCES IN NATURE. If the question were asked, which of two nations would be more enduring, one very arrogant and aggressive, the other more patient and persevering, the instinctive answer would be in favor of the first. If the same question were asked with reference to two microbes, one very virulent and capable of rapidly overwhelming its host, the other less virulent and capable of overpowering its host slowly, the same instinctive response would in all probability be made. The history of man and of microbe alike indicates the reverse is true. Many nations and many microbes have failed to act in obedience to Nature's great law of balance among living things. Some nations, and some microbes even, have defied repeatedly the consequences of the great natural law of biologic balance. The end is inevitably the same. For certain periods they

have seemed to overturn the trite saying that "history repeats itself, historians repeat each other"; but each and every aggressively militant nation, and each and every exceptionally virulent microbe, which may have leaped into hideous notoriety, has sooner or later burned itself out, even as a shooting star blazes in the firmament, fades, and is gone.

It will be necessary to recapitulate the essential steps of the cycle of pathogenism at this point, even though it entail repetition.

Progressively pathogenic bacteria must perform certain steps in definite sequence in order to perpetuate themselves. First, they must reach a suitable host; secondly, they must pass through a suitable portal of entry to underlying tissues in that host; thirdly, they must pass into and multiply vigorously within the tissues; fourthly, the descendants of the initial invaders must escape from the tissues of the host in sufficient numbers to provide for the continuance of the race in other, suitable hosts.

The most precarious step is clearly that one which intervenes between the escape of bacteria from one host, and their arrival in other, succeeding hosts. The microbe runs the gauntlet of the great outside world, just as the hermit crab, which has outgrown its shell, runs the gauntlet of its natural

enemies until it can locate and preëmpt a new shell of proper size. Prodigious numbers of eggs of some of the fish are required to provide for a relatively modest number of adults. Prodigious multitudes of microbes likewise depart from one host, but comparatively few reach new hosts. It is clear that in general those strains of pathogenic microbes that live long enough in a host to provide for the escape of a great multitude of descendants will have an advantage in the struggle for existence. The microbe that becomes too aggressive is doomed to extinction.

If the invading microbes, in other words, overwhelm their host too soon, but few descendants will develop before the host becomes a mausoleum instead of an incubator. It will be recalled that acute miliary tuberculosis, a rapidly progressive infection with the tubercle bacillus, is a radical departure from the usual course of tubercular infection. It is not contagious. The microbes are widely disseminated in the tissues of the victim, but do not succeed in escaping to the outside to reach other hosts.

Effects of Unbalance. Bubonic plague is a disease transmitted by the flea from rodent to ro-

dent, from rodent to man, and from man to man. Sometimes the microbe infects the lungs of man. Then the infection is very properly called "pneumonic plague." Pneumonic plague is transmitted in the cloud of infected droplets coughed up by the victims. The period elapsing between infection and complete prostration is very short, indeed; infected individuals cannot travel very far before they are forced to take to their beds. The escape of the microbe from the primary to the secondary series of hosts is thereby immensely difficult, because of the very short time the freshly invaded individuals can move about. Nurses, doctors, and those in close contact with the stricken are very frequently infected, and many die.

The very savageness of the microbic onslaught is its own undoing. Thus, the great Manchurian epidemic of pneumonic plague which occurred within the last decade did not penetrate beyond the main channels of communication. Travel in Manchuria is relatively slow, and most of the infected individuals were prostrated before they could perform more than a few hours' journey. This factor, rather than the efforts of science, halted the epidemic.

From time to time man has accidentally or deliberately disturbed the balance between living things

in definite places by the introduction of alien species which have no natural enemies. Sometimes an attempt has been made to undo the resulting trouble by the subsequent implantation of a new species. The escape of a pair of gypsy moths in New England, brought from Europe as a curiosity, has cost the eastern United States millions of dollars and the loss of many of its magnificent elms. The English sparrow and the starling have driven away some of our most beautiful songbirds. Fungous diseases, and insects accidentally present upon plants imported from foreign lands, have attached themselves to important domestic foods or shade vegetation with disastrous results. These alien species have at the start no natural enemies, and the inevitable result is an unrestricted growth, until such time as Nature can rally and reëstablish a balance. Man's efforts to reëstablish equilibrium by the deliberate encouragement of enemies to the alien horde are usually less successful than Nature's.

Effect of Introduction of Alien Species. When Australia became fairly well settled by sturdy sons of England, so the story goes, rabbits were introduced to furnish sport for the settlers. Rabbits were selected apparently because they mul-

tiply rapidly. They were turned loose in the uncultivated places. The results exceeded the most sanguine expectations. Provided with ample food and having no natural enemies, they increased like compound interest. Soon they overran entire districts. Nothing green escaped their voracious attack. Hunting became a dire necessity, rather than a pleasure. It is said that some of the more adaptable land animals took to the trees to escape destruction, but this may be an exaggeration. In any event, the first lesson to be learned is never to place a rapidly multiplying animal or insect in a country where conditions for growth are good and where there are no natural enemies.

Results of Attempt to eradicate Alien Species. The second lesson was learned in the attempt to exterminate the plague of rabbits. About the time the rabbits became a material menace, a German professor discovered a peculiar microbe in a sick rabbit, which when injected under the skin caused a rapidly fatal infection. This microbe was named the "Bacillus of Rabbit Septicæmia." The professor staged a demonstration. A minute drop of blood from a dying rabbit was introduced under the skin of a sound rabbit. Within six hours the an-

imal sickened and within twelve hours it was dead. The experiment was repeated again and again. The results were marvels of precision. Inoculation, infection, death, all in half a day. The procedure appeared to be astonishingly simple. What more could be desired? Why not import the professor and his death-dealing bacteria, and clean up the plague of rabbits by turning infected rabbits loose at strategic points and letting Nature and the microbe of rabbit annihilation take their course? The experiment seems to have been tried. Nothing happened. Nothing should have been expected.

Mature and careful study of the bacillus of rabbit plague (known as *Bacillus cuniculicida*, in technical language) revealed a flaw in the process. It was simply this: The natural existence of the rabbit-plague microbe is a relatively peaceful one. It locates as a parasite in the upper respiratory tract, where its descendants can escape and readily reach other rabbits. It lives normally upon good terms with its host, causing no serious discomfort and therefore no reaction on the part of the rabbit which would lead to its dispossession. Like all parasites, it must be agreeable, or at least not too disagreeable, to its host to be tolerated. The germ of rabbit septicæmia, like the Streptococcus and other

parasitic "opportunists" of man, has an inherent and terrible power for harm if it escapes from its normal habitat in the upper respiratory passages into the underlying tissues and the blood-stream. A cut, scratch, or wound is required to furnish this portal of entry to the underlying tissues, however. The virulence of the microbe, it was discovered, can be increased very materially by passing it deliberately from the tissues of one rabbit to the tissues of another rabbit. This excursion into the tissues, however, is inevitably fatal to the microbe, as well as the host.

Nature providentially prevents this extraordinary exaltation of microbic virulence. The sudden, overwhelming infection of the stricken animal takes away all desire to indulge in combat or other violence which would permit of the escape of the microbes from the tissues and the blood-stream of the infected animal to the tissues of other hosts. The microbe has no chance to escape from its unusual and abnormal excursion in the interior of the sick rabbit to the outside to infect other rabbits before death intervenes. This is the reason for the lack, the utter lack, of success in exterminating rabbits from Australia by microbic intervention.

Microbic Balance with Man. Measles is a common disease of childhood — so common that some of our immediate ancestors exposed their offspring to mild cases of the disease with the deliberate expectation of securing a mild attack and lifelong immunity to subsequent infection. It was assumed that practically every child would have measles, and this form of naturally induced immunity was, on the whole, the safest way to avoid contact with a severe case, with the certainty, or almost certainty, of a severe or even fatal attack.

Gradually the white race has become somewhat resistant to measles through a long series of infected generations, and, on the whole, the disease is popularly regarded as distinctly less formidable to life than, for example, scarlet fever. There is reason to believe that the current strains of measles germs (whatever they may be, for they are wholly unknown) have lost some of their deadly severity of infection, and, also, there is reason to believe that the current generations of the Caucasian race, and races in contact with them, have lost some of their early susceptibility to measles. A state of balance exists, as it were, between man and measles virus, not wholly fixed, but at least only moderately variable. It seems logical to assume that the more vir-

ulent strains of measles virus have gradually disappeared. They killed their victims off before the virulent descendants of this type of virus could escape to a sufficient number of successive hosts to perpetuate the strain. On the other hand, the less resistant humans probably have been killed off, thus raising somewhat the average resistance of the race to the measles infection.

When Captain James Cook made his famous voyage to the Pacific, he visited the Sandwich Islands among other places. These islands had never before been invaded by Europeans, and measles was apparently unknown there. A mild case of the disease seems to have existed on one of his ships. The disease was transmitted to the natives, and a most terrible plague it proved to be — quite unlike an epidemic of measles in Europe. The explanation for this extraordinary severity of infection appears to be that measles was an entirely new disease to the Sandwich Islanders. They had no accumulated tolerance, and immunity had to be acquired at one fatal time. Those who survived were in much the same condition as the natives of countries where the balance between microbe and human has been acquired through many generations. Now the Sandwich Islander has no more dread of measles

than the European. A state of balance has been reached between man and measles virus. Other examples of this sudden, almost explosive infection with germs that are not conspicuous between times are quite well known.

One more factor deserves mention: certain kinds of microbes, which appear to be practically identical in their characteristics, mode of infection, and similarity of disease processes, may differ in their reactions with the infected individual in such a manner as wholly to baffle attempts to explain their activities. An example will make this statement clearer.

It is known that recovery from many kinds of infection, such as measles or typhoid fever, may lead to relative or nearly absolute refractoriness to reinfection with the same microbe. In some infections, as typhoid or meningitis (epidemic cerebro-spinal meningitis, it is called technically), the chemical basis for this refractoriness to reinfection is detectable in the blood of the patient. Substances appear during recovery which will kill the invading microbe, but none other. These substances may persist for years after complete convalescence. In some instances, incredibly small amounts of blood from such "immune" patients will actually dissolve the offending germs.

Animals may be inoculated with such germs, using larger and larger doses, until a time comes when very large numbers of the microbe will have no effect upon the animal, although far smaller doses would kill it at the start. The substances which will kill or restrain specific microbes are called "specific anti-bodies." Specific anti-bodies, as diphtheria "antitoxin," may be generated in animals, and the blood of these animals, freed from blood-cells, used for curative purposes by injection into men sick with the same disease.

MENINGOCOCCUS BALANCE. In a similar manner, a specific "anti-meningitis serum" has been prepared which has considerable curative value for man. When this "anti-meningitis serum," as it is called, was tried in human cases in France, some responded very well, whereas some cases — apparently identical — were not benefited in the least. Careful studies by a skilled French scientist revealed the astonishing and wholly unexpected fact that there were at least two kinds of meningococcus germs, both wholly alike except that they made different poisons. This introduced a new problem into the study of microbic disease. It was as if two brothers, both criminal chemists, produced cyanide

of potassium and bichloride of mercury. The antidote for the one is quite different from the antidote for the other. The blood of an animal that had been so treated that it would destroy meningococcus germs of Kind A was wholly, or nearly wholly, ineffective against meningococcus germs of Kind B. The two kinds of antidotes were quite unlike, in other words. When this difference in "anti-body" production was realized, the proper steps were taken to prepare the two kinds of curative serum. When these were tried out, the apparently refractory cases were as readily cured by the second type as those which responded to the first type. Microbes of the same kind, which differ merely in that they do not cause common "anti-body" formation, are called "serologic strains."

Importance of Serologic Balance. The importance of serologic strains is readily grasped. If, for example, a microbe of Kind A is common in one country, a state of balance between it and the humans of that country is very probably to be expected. If, however, Kind B, indigenous in another country with its balance with humans of its own land, is introduced into the territory of Kind A, a very peculiar condition may arise. The clini-

cal disease produced by Kind B will be well understood: it will be a counterpart of that produced by Kind A, but serum that will kill Kind B microbes will be required to effect cures. Only experienced observers will comprehend that an alien "serologic strain" has crossed the frontier.

The question of balance between microbic virulence and human resistance is an entirely new subject. Comparatively little is known about it. Much remains to be learned. This problem is inextricably locked up with the new and important study of "serologic strains." It may be said, however, that the outlook is bright. Man is and always has been more than the peer of the microbe. Otherwise man would have ceased to exist long ago. The gradual workings of the man-microbic balance, therefore, are always against the microbe. Many diseases are slowly dying out. Thus, leprosy has been greatly reduced by natural processes, although up to the Middle Ages it was a thoroughly dreaded pestilence. Advances in the science of Bacteriology are always, even though slowly, reducing the terrors of our hidden foes.

Summary and Conclusion. Mankind is gradually gaining an insight into the wonderful develop-

mental processes which have slowly transformed our Mother Earth from an inconsequential, inorganic speck in space, to a world teeming with life and holding forth almost unlimited possibilities for the future. The effectiveness of these constructive forces of animate nature is dependent upon the summation of a multitude of individually minute contributions. The operation of the chlorophyll granule in the green leaf, the activity of the microbe in the living earth, and the labor of the scholar and scientist alike, is each small in itself, but magnificent in the aggregate. The versatile character of the microbe is strikingly revealed in the contemplation of the kaleidoscopic mosaic of life. With the flight of time, the living tableau is continually changing. The elements from which the fading picture is made are constantly being recast into new and more intricate designs. The prime function of the microbe is to restore these elements in utilizable form to the plant kingdom for reconstruction into new living substance. In this manner is the cycle of life perpetuated.

Even though the pernicious activities of a few microbes are in opposition to the well-being of plant and animal and human life — and some plants and animals and men are equally antagonistic — the

baneful influence of these hidden foes is far outweighed by the constructive and beneficent effects of microbic participation in the life-processes. Science has scarcely crossed the threshold of a new era in which a multitude of unseen but willing microscopic toilers will be drawn from the great living earth to work in the interest of mankind as agriculturists, artisans, and chemists of extraordinary skill. They will perform analyses and syntheses as a matter of routine that would be regarded, and justly regarded, at the present time as miracles.

THE END

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